RNA-BASED THERAPIES

MicroRNA therapeutics: towards a new era for the management of cancer and other diseases

Rajesha Rupaimoole and Frank J. Slack

Abstract | In just over two decades since the discovery of the first microRNA (miRNA), the field of miRNA biology has expanded considerably. Insights into the roles of miRNAs in development and disease, particularly in cancer, have made miRNAs attractive tools and targets for novel therapeutic approaches. Functional studies have confirmed that miRNA dysregulation is causal in many cases of cancer, with miRNAs acting as tumour suppressors or oncogenes (oncomiRs), and miRNA mimics and molecules targeted at miRNAs (antimiRs) have shown promise in preclinical development. Several miRNA-targeted therapeutics have reached clinical development, including a mimic of the tumour suppressor miRNA miR-34, which reached phase I clinical trials for treating cancer, and antimiRs targeted at miR-122, which reached phase II trials for treating hepatitis. In this article, we describe recent advances in our understanding of miRNAs in cancer and in other diseases and provide an overview of current miRNA therapeutics in the clinic. We also discuss the challenge of identifying the most efficacious therapeutic candidates and provide a perspective on achieving safe and targeted delivery of miRNA therapeutics.

Non-coding RNA
Naturally transcribed RNA molecule that does not encode any protein. Family members include microRNAs and long non-coding RNAs.

miRNA mimics
(MicroRNA mimics). Synthetically derived small RNA molecule duplexes, which, upon introduction into the cells, behave similarly to endogenous miRNAs.

Institute for RNA Medicine, Department of Pathology, Beth Israel Deaconess Medical Center Cancer Center, Harvard Medical School, Boston, Massachusetts 02215, USA.

Correspondence to F.J.S.
fslack@bidmc.harvard.edu

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MicroRNAs (miRNAs) are a class of non-coding RNA molecules that play a central part in cell differentiation, proliferation and survival by binding to complementary target mRNAs, resulting in mRNA translational inhibition or degradation[1]. The first miRNA was identified in 1993 as a small RNA transcribed from the Caenorhabditis elegans lin-4 locus[2], and 7 years later the first mammalian miRNA, let-7, was discovered[3]. These two key events led to a series of genomic investigations that revealed extensive transcription of many miRNAs and other non-coding RNAs[1–5].

The functional validation of these transcripts has enabled a better understanding of cellular and developmental biology and of various diseases at the molecular level[1,5–11]. For example, loss of *lin-4* or *let-7* in *C. elegans* results in severe developmental defects, including aberrant cell fate specifications, indicating that miRNAs are key players in development[2,3,12]. The initial concept of miRNAs as developmental regulators has now substantially expanded, and miRNAs are found to be dysregulated in numerous diseases, including cancer, hepatitis and cardiovascular diseases[1,5,8,10]. miRNAs are frequently altered in disease owing to genomic events, such as mutations, deletion amplification or transcriptional changes, or to biogenesis defects due to mutations or the downregulation of enzymes that regulate miRNA biogenesis[1,10,13,14] (FIG. 1).

In humans, the biogenesis of miRNAs involves tightly regulated pathways involving four key enzymes — Drosha, exportin 5, Dicer and argonaute 2 (AGO2)[1,13] — which are described in detail in FIG. 1. Mutations in genes encoding biogenesis pathway-related enzymes such as Dicer, Drosha, exportin 1 and AGO2 occur in numerous cancer types, including neuroblastoma, ovarian cancer and Wilms tumours[10,14–18].

The ability of carefully selected miRNAs to target multiple mRNAs that are altered in disease conditions makes these molecules interesting candidates as therapeutics (in the form of miRNA mimics) or as targets of therapeutics (in the form of antimiRs)[8,10,19,20] (BOX 1; TABLE 1). In parallel, advances in technologies to deliver RNA molecules (BOX 2) *in vivo* have made miRNA-based therapeutics feasible. These constructs have various modifications in their RNA backbone to provide higher stability and protection from nucleases (BOX 1; FIG. 2).

In initial studies, naked miRNA mimics, or miRNA mimics encoded in viral vectors, were injected either systemically or locally at target tissue sites. However, owing to pharmacological challenges such as degradation in the bloodstream and poor delivery to the target site of systemically delivered miRNA mimics, as well as the clinical difficulties associated with local delivery, the initial studies resulted in little success in moving

AntimiRs
Also called microRNA (miRNA) inhibitors, antimiRs are small, synthetically derived molecules, which have sequence complementary to target mature miRNAs. They are known to sequester target miRNAs and are used to suppress miRNA function.

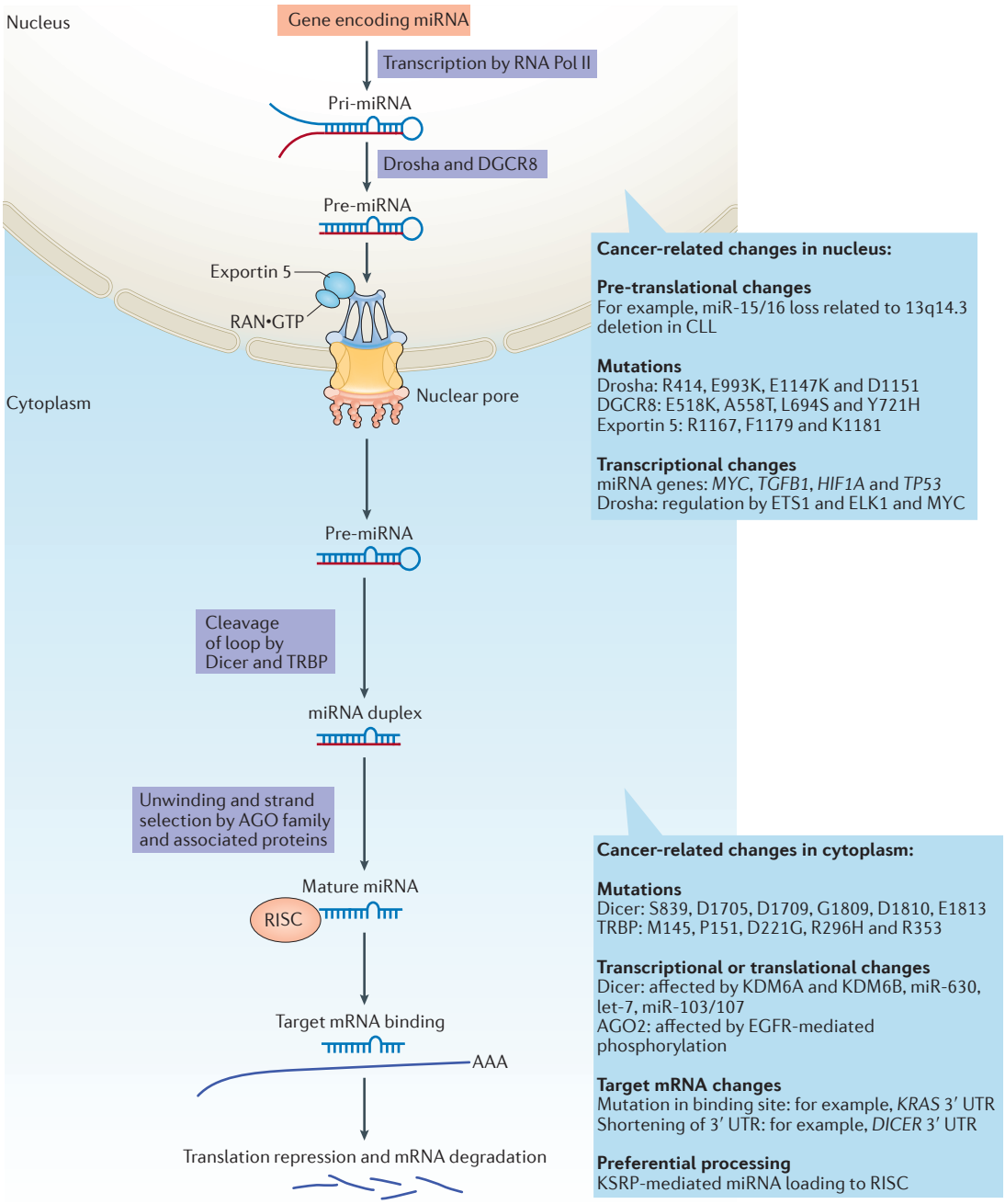

Figure 1 | **miRNA biogenesis.** Overview of microRNA (miRNA) biogenesis, highlighting key mutations and deregulated factors that play a part in diseases related to alterations in miRNAs. miRNAs are produced in a tightly regulated pathway that is conserved across species[1,13]. The biogenesis of miRNA begins with their transcription by RNA polymerase II (Pol II). The majority of genes encoding miRNAs are located in intronic regions and contain their own promoter regions. Following RNA Pol II-mediated transcription of long primary transcripts, the first of two enzymatic cleavages that produce mature miRNAs commences. Drosha, a type III RNase, along with the cofactor protein DGCR8, binds to the primary miRNA (pri-miRNA) transcript. Two RNase domains that are present in Drosha mediate the cleavage of the 3′ and 5′ strands of pri-miRNAs to generate pre-miRNA. Next, the exportin 5–RAN•GTP complex mediates the movement of pre-miRNAs from the nucleus into the cytosol. There, the RNase III Dicer and TAR RNA binding protein (TRBP) bind to the pre-miRNAs and cleave the terminal loop, resulting in a miRNA duplex. In the next step, the miRNA duplex is incorporated into the RNA-induced silencing complex (RISC). Processing of the miRNA duplex is mediated by the argonaute (AGO) family of proteins, in conjunction with several cofactors such as PACT (also known as PRKRA). Following unwinding and strand selection, the mature miRNA is capable of target recognition. Binding of the mature miRNA to RISC leads to the targeting of mRNAs with complementary sites and results in translational repression or mRNA degradation. Mutations in genes encoding biogenesis pathway-related enzymes such as Dicer, Drosha, exportin 1 and AGO2 have been reported in numerous cancer types, including neuroblastoma, ovarian cancer and Wilms tumours, as highlighted in the figure[10,14–18]. CLL, chronic lymphocytic leukaemia; EGFR, epidermal growth factor receptor; KDM, lysine-specific demethylase; KSRP, KH-type splicing regulatory protein; UTR, untranslated region.

Box 1 | **Chemical modifications of miRNA-based therapeutics**

MicroRNA (miRNA)-based therapeutics can be divided into miRNA mimics and inhibitors of miRNAs (also known as antimiRs). miRNA mimics are synthetic double-stranded small RNA molecules that match the corresponding miRNA sequence and therefore functionally aim to replenish the lost miRNA expression in diseases. By contrast, antimiRs are single stranded and based on first-generation antisense oligonucleotides (ASOs), which had been designed to target mRNAs, or modified with locked nucleic acids (LNAs). AntimiRs with a 2′-*O*-methoxyethyl modification are also called antagomiRs. These synthetic small RNA molecules have a complementary sequence to the miRNA to be inhibited and block the function of the corresponding miRNA by binding to it strongly. Over the years, significant improvements in binding affinity, stability and target modulation effects of miRNA mimics and antimiRs have been achieved through chemical modifications to the nucleotide backbone.

One of the challenges for RNA-based therapeutic strategies (including single- or double-stranded oligonucleotides) is the potential for degradation of oligonucleotides by RNases in serum or in the endocytic compartment of cells. To avert the issue of degradation inside cells, two different yet converging strategies have been investigated. One is to alter oligonucleotide chemistry by modifying the nucleotides or the RNA backbone through methylation or LNAs, or by adding phosphorothioate-like groups. A second strategy is to develop delivery vehicles to encapsulate RNAs for protection and allow endosomal escape (BOX 2; FIG. 2). Currently available commercial miRNA mimics are often modified by methylation of the passenger strand for increased stability, and antimiRs are modified using LNA chemistry. However, most of the effort towards developing chemically modified miRNA therapeutics is dedicated to the development of antimiRs.

ASOs

First-generation ASOs were modified by replacing the non-bridging oxygen in the phosphate group with sulfur, thereby generating phosphorothioate nucleotides. This modification increases the stability of ASOs inside cells (by making internucleotide linkages resistant to nucleases degradation) while retaining sufficient RNase H activation for mRNA target cleavage and function in suppressing target gene expression. Additional modifications of ASOs that have been tested include the addition of methyl groups at different locations in the RNA backbone. The addition of a 2′–*O*-methyl group to phosphorothioate nucleotides resulted in increased binding affinity to target mRNA, significant nuclease resistance and higher *in vivo* stability[168]. A 2′-*O*-methoxyethyl modification also improved nuclease resistance and binding affinity[168]. Based on this second-generation chemistry, several ASOs have moved to various phases of clinical trials, including a commercialized ASO to treat patients with homozygous familial hypercholesterolaemia (Mipomersen, an ASO against mRNA of apolipoprotein B developed by Ionis Pharmaceuticals).

AntimiRs

AntimiRs are structurally similar to ASOs. AntimiRs are designed to bind directly to the mature strand of the targeted miRNA and thus to induce a functional blockade. Recent studies have investigated different types of modifications of antimiRs that had previously been developed for ASOs. For example, an antimiR with a 2′-*O*-methoxyethyl modification against miR-122 resulted in improved target modulation compared with unmodified antimiRs[169]. Furthermore, LNA-modified antimiRs have significantly advanced the oligonucleotide chemistry field. LNA-modified antimiRs are chemically locked by a bridge that connects the 2′-oxygen and 4′-carbon in a ribonucleotide, mimicking C3′-endo conformation. To enhance the efficacy of miRNA targeting, repeated patterns of two deoxyribonucleotides, followed by one locked ribonucleotide (called LNA mixmers) have been designed, and these mixmers showed promising results *in vivo* in mouse models of cancer, cardiac disease and diabetes, and in non-human primates[124,125].

Recently, our laboratory reported a peptide backbone modification of nucleic acids that is designed to improve tumour delivery[85,119]. We reported the use of a pH low insertion peptide (pHLIP)-modified antimiR to inhibit the oncomiR miR-155 in lymphoma[119]. The peptide antisense nucleotide (containing intramolecular amide linking nucleotides) against miR-155 was modified by the addition of pHLIP using a disulfide bond. These conjugated peptide nucleic acids enter cancer cells *in vivo* by taking advantage of the low pH in the tumour microenvironment via a non-endosomal route. Under low-pH conditions, such as in the tumour microenvironment, a pH-dependent conformational change, driven by the protonation of aspartic acid residues in the pHLIP, results in the insertion of its carboxyl terminus in the cell membrane to form a transmembrane α-helix[170]. Upon insertion into cells, the release of cargo (such as antimiR-155 present on the carboxyl terminus) is facilitated by the cleavage of the disulfide bond in the cytosol[119,170].

these approaches into the clinic[20–22]. Advances in RNA chemistry (BOX 1) and delivery technologies, including nanoparticle systems (BOX 2), have now enabled the first miRNA-based agents to move into the clinic (FIG. 2; TABLES 1,2).

In this Review, we discuss the recent discoveries related to miRNA alterations in cancer, cardiovascular diseases, hepatitis, atherosclerosis, diabetes and scleroderma. Furthermore, we describe obstacles and advances in the development of miRNA therapeutics, provide an overview of clinical trials involving miRNA mimics and antimiRs, and discuss the future of such therapies.

The role of miRNAs in cancer

There is considerable evidence to indicate that miRNAs and their biogenesis machinery are involved in the development of cancer. Here, we discuss some prominent examples.

Dysregulation of miRNA biogenesis enzymes

The miRNA biogenesis proteins Drosha and Dicer are downregulated in several cancer types and this downregulation has been associated with poor patient outcomes[23–29]. *DROSHA* expression is regulated by potentially oncogenic transcription factors such as MYC[25] or the RNA-specific

deaminase ADARB1, leading to decreased primary miRNA (pri-miRNA processing[26]. Recently, Drosha was also reported to be downregulated in response to tumour hypoxia, and this process was mediated by the direct binding of the hypoxia-responsive transcription factors ETS1 and ELK1 to the promoter of *DROSHA*.

The mechanisms of Dicer downregulation in cancer are highly diverse. For example, Dicer downregulation can be due to the downregulation or loss of the transcription factor TAp63, which is a frequent occurrence in cancer. TAp63 normally activates *DICER* expression by directly binding to its promoter[30]. Dicer can also be

Table 1 | **Selected miRNAs in cancer and other diseases and their therapeutic manipulation in preclinical models**

miRNAs	Diseases	Important mRNA targets	Preclinical models	*In vivo* delivery systems
miRNAs with tumour suppressive function (miRNA mimics as therapeutics)				
let-7 family	• Solid tumours (e.g. breast, colon, ovarian, lung, liver and glioma) • B cell lymphoma	*MYC*, *BCLXL*, pan-*RAS*, *EZH2*, *HMGA2*, *FAS*, *P21*, *PGRMC1* and *DICER1*	• Lung cancer (orthotopic)[107] • *Kras*^{G12D} GEM[109]	Neutral lipid emulsions
miR-34a	• Solid tumours (e.g. lung, liver, colon, brain, prostate, pancreatic, bladder and cervical) • Myeloma • B cell lymphoma	*BCL2*, *MET*, *MYC*, *CDK6*, *CD44*, *SRC*, *E2F1*, *JAG1*, *FOXP1*, *PDGFRA*, *PDL1* and *SIRT1*	• Lung cancer (xenograft and orthotopic)[107] • *Kras*^{G12D} GEM[109] • Pancreatic cancer (orthotopic)[108] • Prostate cancer (orthotopic)[37]	• Lipid nanoparticles • Neutral lipid emulsions
• miR-143 • miR-145	• Solid tumours (e.g. bladder, lung, breast, colon, pancreas, cervical, and head and neck) • Lymphoid leukaemia	*KRAS*, *ERK5*, *VEGF*, *NFKB1*, *MYC*, MMPs, *PLK1*, *CDH2* and *EGFR*	• Colon cancer (orthotopic)[190] • Pancreatic cancer (orthotopic)[191]	Liposomes, PEI
miR-200 family	Solid tumours (e.g. breast, ovarian and lung)	*ZEB1*, *ZEB2*, *BMI1*, *SUZ12*, *JAG1*, *SOX2*, *SP1*, *CDH1* and *KRAS*	• Lung cancer (orthotopic)[61,111] • Ovarian cancer (orthotopic)[61] • Breast cancer (orthotopic)[61]	• Liposomes • DOPC neutral lipid system
OncomiRs (antimiRs as therapeutic agents)				
miR-10b	Solid tumours (e.g. breast and glioma)	*NF1*, *CDH1*, *E2F1*, *PIK3CA*, *ZEB1* and *HOXD10*	• Glioblastoma (orthotopic)[116] • Breast cancer (orthotopic)[101]	Locked nucleic acid antimiRs
miR-155	• Solid tumours (e.g. liver, lung, kidney, glioma and pancreas) • B cell lymphoma • Lymphoid leukaemia	*SHIP*, *SPI1*, *HDAC4*, *RHOA*, *SOCS1*, *BCL2*, *JMJD1A*, *SOX6*, *SMAD2*, *SMAD5* and *TP53INP1*	Lymphoma miR-155 overexpressing GEM[185, 119]	pHLIP-conjugated antimiR
• miR-221 • miR-222	Solid tumours (e.g. liver, pancreas and lung)	*CDKN1B*, *CDKN1C*, *BMF*, *RB1*, *WEE1*, *APAF1*, *ANXA1* and *CTCF*	Liver cancer (HCC xenograft)[118]	Cholesterol-conjugated antimiR
Other				
miR-122	HCV infection and related liver diseases	HCV 5′ site, *CAT1*, *CD320*, *ALDOA* and *PPARB*	HCV mouse model[124,128]	Phosphorothioate DNA–locked nucleic acid antimiR
miR-33	Atherosclerosis	*SREBF2*, *ABCA1*, *CROT*, *CPT1A*, *HADHB* and *PRKAA1*	HFD mouse[143,144]	• 2′-F or MOE phosphorothioate DNA antimiR • Locked nucleic acid antimiR
miR-208	• Cardiac disease • Cardiac stress • Myocardial infarction	*MED13*, *SOX6* and *MYH7B*	Dahl hypertensive rat[139]	Locked nucleic acid antimiR
miR-21	• Kidney fibrosis • Cardiac fibrosis	*PTEN*, *PDCD4*, *SMAD7*, *SPRY* and *PPAR*	• Pressure overload model of heart disease[130] • Kidney injury mouse model[131]	Locked nucleic acid antimiR
miR-192	Diabetes-related kidney complications	Type I collagens, *ZEB1* and *ZEB2*	Streptozotocin-induced type 1 diabetes mouse[192]	Locked nucleic acid miRNA mimic
miR-29c	Diabetes-related kidney complications	*HDAC4* and MMPs	*db/db* mouse[193]	Naked antagomiRs
• miR-103 • miR-107	Diabetes	*CAV1*	• *ob/ob* mouse[156] • HFD mouse	Locked nucleic acid antimiR
miR-15	Myocardial infarction	*CHEK1*	Ischaemia–reperfusion injury mouse[194]	Locked nucleic acid antimiR

2′-F, 2′-fluoro; *db/db*, spontaneous diabetes due to a mutation in the leptin receptor gene; *ob/ob*, spontaneous diabetes due to a mutation in the leptin gene; DOPC, 1,2 dioleoyl-*sn*-glycero-3 phosphatidylcholine; GEM, genetically engineered mouse; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HFD, high-fat diet; PEI, polyethylenamine; miRNA, miR, microRNA; MMPs, matrix metalloproteinases; MOE, 2′-*O*-methoxyethyl; pHLIP, pH low insertion peptide.

downregulated through direct targeting of the *DICER* 3′ untranslated region (UTR) by miRNAs such as miR-103/107 (REF. 31), *let-7* (REF. 32) and miR-630 (REF. 33). Interestingly, tumour hypoxia further influences these effects. Downregulation of *DICER* expression by epigenetic mechanisms, which are mediated by the hypoxia-induced inhibition of the oxygen-dependent trimethylated histone H3 lysine 27 (H3K27me3) demethylases KDM6A and KDM6B[34], is one such event.

The miRNA biogenesis protein AGO2 can be inhibited in cancer by epidermal growth factor receptor (EGFR)-dependent phosphorylation[35]. Breast cancer cells exposed to hypoxia have an increased association between EGFR and AGO2, leading to the phosphorylation of AGO2 at the Y393 residue. This process results in decreased AGO2 binding to Dicer, functionally resulting in increased cancer cell survival and invasiveness.

Moreover, mutations in the gene encoding exportin 5 have a key role in decreasing the cytosolic export of miRNAs in cancer. This effect results in the increased expression of oncogenes such as *EZH2* and *MYC* owing to the release of the suppressive effects of miRNAs on their expression[18].

In addition to the direct deregulation of miRNA biogenesis, the DNA damage response in cancer cells can lead to increased processing of selected sets of miRNAs. This effect is due to the ATM kinase-dependent phosphorylation of KH-type splicing regulatory protein (KSRP)[36], which results in the binding of KSRP to pri-miRNAs and their subsequent preferential processing. The functional effects of such preferential processing remain unclear. However, we speculate, based on the downregulation of KSRP in cancers, that a decrease in such preferential processing may increase tumorigenesis due to the loss of tumour suppressor miRNAs.

Dysregulation of tumour-suppressive miRNAs

The miR-34 family of miRNAs. Among the miRNAs dysregulated in cancer, the miR-34 family has received substantial attention, with three members, miR-34a, miR-34b and miR-34c, downregulated in lung, breast and many other cancers37–40. All three family members are transcriptionally regulated by the tumour suppressor p53 during the DNA damage response, and p53 and the DNA damage response are often altered in cancer cells39,41–43. miR-34a, one of the best-studied members of the family, plays a part in p53-mediated apoptosis upon DNA damage by directly targeting the anti-apoptotic protein sirtuin 1 (SIRT1)40. The tumour suppressive role of miR-34 is also evidenced by its target mRNA network, affecting the expression of cell cycle proteins such as cyclin-dependent kinase 4 (CDK4) and CDK6, anti-apoptotic proteins such as BCL-2 and metastasis-related proteins such as MET, Notch, MYC and AXL[44].

In recent years, studies have demonstrated the role of programmed cell death protein 1 (PD1) and PD1 ligand 1 (PDL1) in immune invasion by cancer cells[45]. Increased expression and interaction of PD1 and PDL1 have a significant negative role in disease progression. In acute myeloid leukaemia (AML)[46] and non-small-cell lung cancer (NSCLC)[47] cell lines, miR-34 downregulates the expression of PDL1, indicating an alternative pathway by which p53 modulates immune evasion in cancer. The authors further showed a tight inverse correlation between miR-34 and *PDL1* expression[47].

Given the large pool of oncogenic mRNAs that are downregulated by miR-34, much attention has focused on therapeutics designed to replenish miR-34, which have progressed into clinical trials[19] (see below).

The let-7 family of miRNAs. Several studies have demonstrated that loss of let-7 has a causative role in various cancers[7,12,32,48–52]. In humans, the let-7 family includes ten isoforms that target a wide range of mRNAs encoding oncogenes[7]. These oncogenes include *KRAS*, a proto-oncogene that is often activated in cancer[53]. The biogenesis of let-7 is controlled by mechanisms such as direct targeting of pre-let-7 by the miRNA-binding protein LIN28 (REF. 54) or by methylation of the promoter region of the *let-7* gene, mediated by the methyltransferase DNMT1 (REF. 50). Loss of let-7 in cancer cells results in accelerated tumour progression owing to perturbations of signalling networks involving the RAS family of proteins[53]. A negative feedback loop in which let-7a targets the 3′ UTR of *DICER* has been reported in NSCLC[32]; however, the functional implications are not fully elucidated. In breast cancers, let-7 regulates cancer stem cell self-renewal and differentiation by downregulating the expression of *HRAS* and the transcriptional cofactor *HMGA2*, respectively[48]. These data suggest that therapeutic strategies to replenish let-7 may be of use in cancers in which let-7 is lost or downregulated.

The miR-200 family of miRNAs. Another important set of miRNAs that is downregulated in cancer is the miR-200 family, which modulates the expression of proteins involved in tumour metastasis and angiogenesis. The human miR-200 family consists of two groups, miR-200a/b/429 and miR-200c/141, located on chromosome 1 and chromosome 12, respectively[55]. Despite the difference in chromosomal locations and different expression patterns between the two groups, their targets and biological functions overlap substantially, mainly involving factors that play a part in epithelial–mesenchymal transition (EMT). The EMT programme in cancer cells has been associated with increased cancer metastasis because the change in phenotype from epithelial to mesenchymal allows cancer cells to become invasive[56]. miR-200 directly downregulates the transcriptional inhibitor zinc-finger E-box-binding homeobox 1 (ZEB1), a known transcriptional repressor of cytoskeletal rearrangement protein E-cadherin (also known as CDH1), thereby promoting EMT during cancer metastasis[55,57,58]. In addition to miR-200 directly targeting *ZEB1* mRNA, a negative feedback loop regulating miR-200 expression was identified, whereby ZEB1 binds to the promoter of *mir-200*, resulting in reduced *mir-200* expression[59]. Another factor that downregulates *mir-200* expression is transforming growth factor β1 (TGFβ1)[60], a cytokine that also promotes EMT. In addition to the role of miR-200 in EMT,

Box 2 | **Delivery systems for miRNA therapeutics**

Enhancing the stability of microRNA (miRNA) mimics under *in vivo* conditions using chemical modifications has limitations, and one such limitation is the loss of mRNA silencing ability. This loss of efficiency is due to loading of the miRNA into the RNA-induced silencing complex (RISC). This limitation has led to the development of alternative approaches to increase the efficacy of *in vivo* delivery, such as encapsulating the miRNA mimic into nanoparticles. Considering the similarity between miRNA mimics and small interfering RNA (siRNA) structure and functions (both are double-stranded small RNA molecules), knowledge gained from the development of siRNA delivery methods, some of which are now in late-stage clinical trials, can inform the development of delivery methods for miRNA therapeutics.

Viral vectors
Adenoviral vectors that encode small RNA molecules of interest have been constructed; however, it remains challenging to bring this method to the clinic owing to safety issues[22].

Poly(lactide-co-glycolide) particles
Poly(lactide-co-glycolide) (PLGA) is a polymer that is widely used for the delivery of small RNAs *in vivo*. PLGA has low toxicity owing to its neutral charge, and the delivery rate of RNA molecules can be controlled by altering the composition of the PLGA particles[171,172]. PLGA has been used in the clinic for biodegradable sutures, and it has a high safety profile. However, limitations include the low rates of siRNA or miRNA loading. One study used a double-emulsion technique combining amphiphilic cationic lipid BHEM-cholesterol and PLGA particles, which was shown to increase the siRNA incorporation efficiency[173].

Neutral lipid emulsions
Among the lipid-based delivery systems, neutral lipid emulsions (NLEs) constitute a significant proportion of tested vehicles. NLEs consist of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), squalene oil, polysorbate 20 and an antioxidant. NLEs are neutral charge nanoparticles with low toxicity[107] but have limitations with regard to the efficiency of delivery to tumour sites.

Neutral liposome 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine
DOPC-based nanoparticles have been widely used in the preclinical setting for the delivery of siRNAs[68,69,174] and have advanced to phase I trials for siRNA-based approaches: for example, siEphA2 by the University of Texas MD Anderson Cancer Center, USA (clinicaltrials.gov identifier: NCT01591356). These nanoparticles have been tested in preclinical studies to deliver miRNA mimics[33,61,65,175].

EnGeneIC Delivery Vehicle nanocells
EnGeneIC Delivery Vehicle (EDV) nanocells (also called TargomiRs) are bacterium-derived 400 nm particles that had previously been shown to have the capacity to deliver chemotherapeutic agents and have been modified with surface-conjugated antibodies to enable specific targeting of disease sites[176,177]. EDV nanocells coated with epidermal growth factor receptor (EGFR)-specific antibodies are currently in a phase I trial for the delivery of miR-16 mimics (clinicaltrials.gov identifier: NCT02369198).

Synthetic polyethylenimine
Polyethylenimine (PEI) is one of the early-generation polymers studied for nucleic acid delivery[178]. Upon forming a complex with nucleic acids, PEI retains a small positive charge, which allows it to adhere to the negatively charged cell membrane and undergo endocytosis. *in vivo*-jetPEI is a commercially available delivery system comprising PEI particles that is currently being tested in preclinical development for delivery of siRNAs and miRNAs[178].

Dendrimers
Dendrimers consist of poly(amidoamine)- or poly(propylenimine)-conjugated nucleic acids. These molecules have shown a high efficiency in delivering nucleic acids such as siRNAs in mouse studies; however, owing to their cationic charge, they are often associated with toxicity[179].

Cyclodextrin
This glucose polymer has been widely used in medical formulations[180]. The first clinical trial of an siRNA therapeutic used cyclodextrin-based delivery. Significant mRNA target engagement was shown; however, the trial was terminated owing to dose-limiting toxicity[181].

Poly(ethylene glycol)
One of the most advanced siRNA delivery systems currently in clinical trials is based on poly(ethylene) (PEG)–siRNA conjugates, in which nucleic acids are conjugated to PEG via a disulfide linkage[182]. These particles showed superior gene-silencing efficacy compared with the PEI system. These conjugates were further modified by linkage to cyclodextrin, and the resulting molecules were used in the first clinical trial involving siRNAs[181].

Chitosan
Chitosan is a cationic polymer derived from chitin (a naturally occurring polysaccharide composed of glucosamine and *N*-acetylglucosamine residues) and has been extensively used for the delivery of siRNAs in preclinical studies[183]. Owing to their biodegradability and low cellular toxicity, chitosan–nucleic acid conjugates provide an attractive platform for delivering miRNAs.

***N*-acetyl-D-galactosamine**
siRNAs or miRNA mimics can be conjugated to *N*-acetyl-D-galactosamine (GalNAc), which leads to their uptake into cells by clathrin-mediated endocytosis. GalNAc–siRNA conjugates, such as ALN-PCSsc (Alnylam Pharmaceuticals), and GalNAc–miRNA conjugates, such as RG-101 (Regulus), are currently being evaluated in phase I and phase II trials. An advantage of GalNAc conjugates is that they can be delivered without the need of additional delivery carrier molecules such as lipids[184]. GalNAc–siRNA conjugates efficiently accumulate in the liver owing to their high affinity for the asialoglycoprotein receptor. However, this modification limits the use of GalNAc chemistry beyond hepatocytes related altered gene diseases. Some of the current clinical trials with siRNA formulations containing GalNAc are highlighted in Supplementary information S1 (table).

it has a role in angiogenesis: loss of miR-200 results in an increased expression of its targets interleukin-8 and C-X-C motif chemokine 1 (CXCL1) in cancer-associated endothelial cells, both of which are chemokines that promote angiogenesis[61].

miR-15/16. The chromosomal locus 13q14.3 is often deleted in chronic lymphocytic leukaemia (CLL), and within this region lies the tumour suppressor miRNA cluster miR-15/16 (REF. 62). A causative relationship between miR-15/16 loss and CLL development was demonstrated in a mouse model[63]. Deletion of the 13q14 minimally deleted region, comprising the miR-15/16 miRNA coding gene[63], resulted in the development of an autonomous lymphoproliferative disorder in mice, a condition that is similar to CLL in humans[63]. Apart from CLL, the miR-15/16 cluster is reported to be downregulated in solid tumours such as bladder cancer, colon cancer and melanoma[62]. Prime targets of miR-15/16 include proteins such as BCL-2, CDC2 (also known as CDK1), ETS1 and JUN, all of which are involved in cancer progression[62].

miR-506. In ovarian cancer, an integrated network analysis of potential mRNA target expression revealed miR-506 as one of the miRNAs that is significantly downregulated in tumours compared with normal ovarian tissues[64]. Moreover, the downregulation of miR-506 promotes metastasis[64]. Studies have discovered a large number of mRNAs that are targets for miR-506. These mRNAs include those that encode proteins that are involved in the DNA damage response (for example, RAD51, a protein involved in double-stranded DNA repair)[65], cellular senescence (for example, CDK4 and CDK6, proteins that regulate the cell cycle)[66] and metastasis (for example, SNAI2, a transcription factor inhibiting EMT signalling)[64].

miR-520. In breast and ovarian cancers, miR-520 is downregulated and appears to act as a tumour suppressor[67,68]. In breast cancer, miR-520 downregulates the expression of *TGFBR2*, which encodes a TGF signalling receptor protein that can promote metastasis[67]. Another direct target of miR-520 is ephrin type B receptor 2 (EphB2)[68]. Ephrin signalling in ovarian cancer results in pro-oncogenic changes that lead to increased tumour growth[69], and suppression of ephrin signalling by miR-520 results in significant tumour reduction in mouse models of ovarian cancers[68].

Dysregulation of miRNAs with oncogenic function

miR-21. Numerous cancer studies have shown that miR-21 has an anti-apoptotic role and is significantly upregulated in tumours compared with normal tissues[70–73]. A role for miR-21 as an oncogenic miRNA (oncomiR) *in vivo* was demonstrated by using a doxycycline-inducible *mir-21*$^{LSL-Tet\text{-}off}$ mouse model[74]. Upon induction of miR-21, the mice developed a malignant pre-B cell lymphoid-like phenotype. A parallel study demonstrated a pro-tumorigenic role of miR-21 in NSCLC[75]. In the *KRAS*^{LA2} model of NSCLC, deletion of *mir-21* resulted in reduced tumorigenesis, whereas overexpression of *mir-21* in this model resulted in increased tumorigenesis.

An amplification of the chromosomal 17q23.2 region, which includes *mir-21*, has been observed in breast, lung, hepatocellular, ovarian and prostate cancers[76]. A recent report analysing lung adenocarcinoma sequencing data from The Cancer Genome Atlas (TCGA) demonstrated that the locus containing the *mir-21* gene is amplified, and that amplification in this genomic region acts as a prognostic marker[77]. However, transcription factors have also been attributed to the increased expression of *mir-21* in cancer. For example, the transcription factor AP-1, which is frequently upregulated in cancer, binds to the *mir-21* promoter[78].

Other important factors involved in miR-21 upregulation are TGFβ1 (REF. 73) and the transcription factor signal transducer and activator of transcription 3 (STAT3)[79]. TGFβ1-mediated stimulation of its receptor TGFβR1 leads to the activation of the transcription factors SMAD2 and SMAD3, which promote the formation of cancer-associated fibroblasts. SMAD7, a known inhibitor of the above signalling cascade, is a direct target of miR-21, and increased levels of miR-21 in normal fibroblasts were associated with transformation of these cells into cancer-associated fibroblasts[73]. The upregulation of *mir-21* transcription under inflammatory conditions in cancer is mediated by STAT3 activation through interleukin-6 (REF. 79). One of the predominant miR-21 targets is programmed cell death protein 4 (PDCD4), a protein involved in apoptosis and metastasis, and its expression is decreased in several cancer types[70,71]. PDCD4 downregulates the expression of p21, CDK4 and the JUN amino-terminal kinase (JNK)–AP-1 pathway proteins, thus affecting apoptosis, cell cycle and cancer cell invasion. Other proteins that are downregulated by miR-21 are reversion-inducing cysteine-rich protein with Kazal motifs (RECK), a protein that inhibits matrix metalloproteinase signalling, maspin (also known as serpin B5), a protein with pro-apoptotic function, and PTEN, a tumour suppressor involved in phosphoinositide 3-kinase (PI3K) signalling[76].

miR-155. Another important tumour-promoting miRNA is miR-155, which acts as a powerful oncomiR in lymphoma and in several types of solid tumours[76,80–85]. miR-155 downregulates the expression of *SHIP1* (also known as *INPP5D*), which encodes a modulator of immune responses, WEE1 G2 checkpoint kinase (*WEE1*), which encodes a cell cycle regulator that plays an important part during DNA damage responses, and many other genes involved in cell homeostasis[81,82,84]. In addition, miR-155 downregulates the expression of von Hippel–Lindau tumour suppressor (VHL), a protein involved in the cellular response to hypoxia. Downregulation of VHL leads to increased angiogenesis and facilitates cancer cell survival[86]. In xenograft mouse models of pancreatic cancer, tumour protein p53 inducible nuclear protein 1 (TP53INP1), a protein involved in pro-apoptotic responses upon p53 activation, was shown to be directly downregulated by miR-155 (REF. 80).

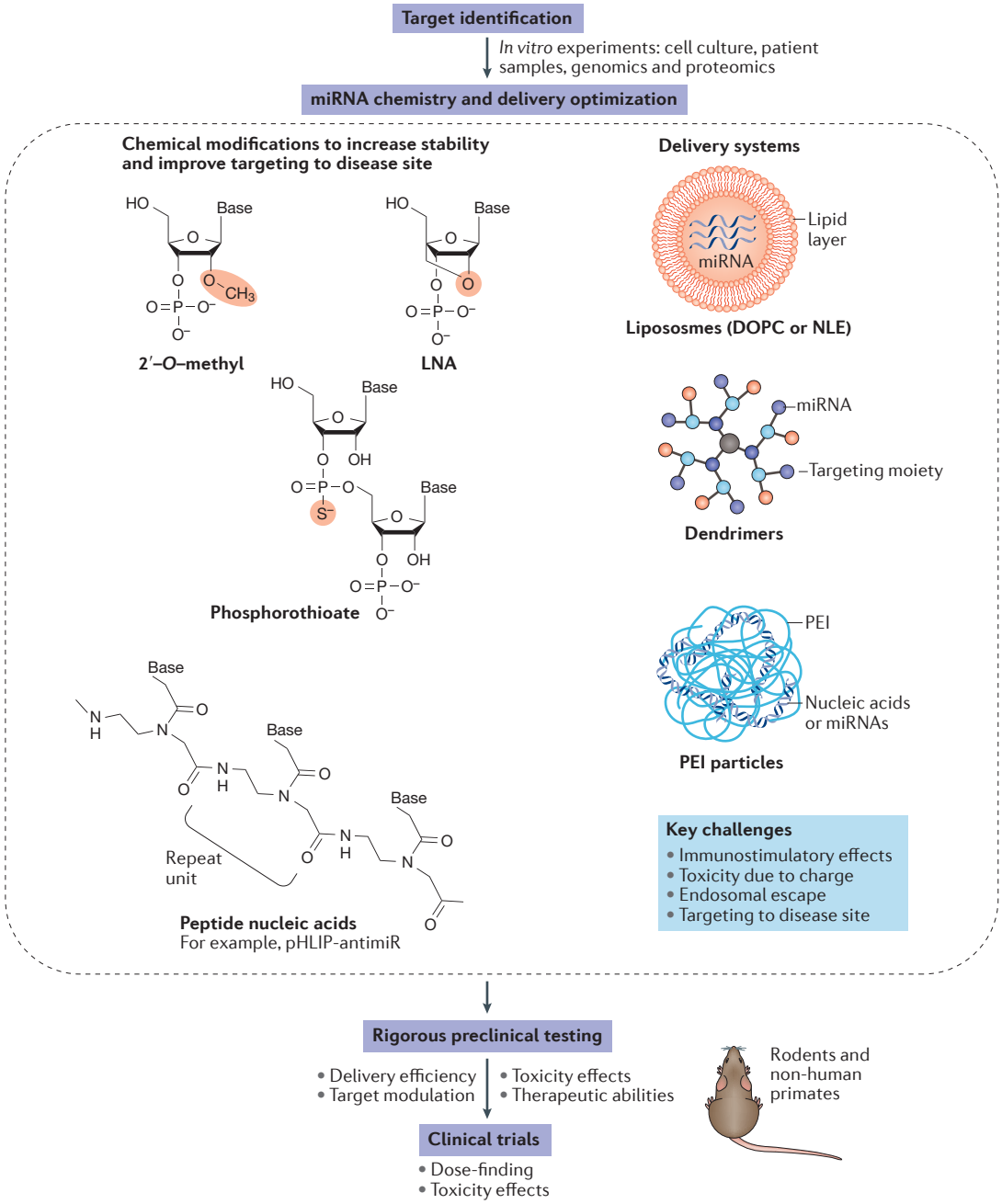

Figure 2 | **Summary of the key steps in the development of miRNA therapeutics.** The first step in the development of microRNA (miRNA) therapeutics involves the systematic selection of miRNA candidates by analysing patient samples and then elucidating the biology and relevance of the miRNA candidates to disease using tissue culture and *in vivo* model-based validation. Currently, there are several public databases that contain genomic and proteomic data from various healthy and diseased tissues. Combining these data with biological validations may facilitate the identification of promising candidate miRNAs. The next major challenge involves the development of chemical modifications and delivery systems for miRNA mimics and antimiRs for *in vivo* applications. One of the major issues for ribonucleotide-based therapeutics is degradation by nucleases and endosomal escape (escape from the endosomal compartment during internalization without degradation). Stability can be increased significantly by chemical modifications such as the addition of a 2′-*O*-methyl group or locked nucleic acids (LNAs). In addition to chemical modifications to small RNA molecules, several encapsulation methods have been developed, resulting in improved delivery to disease sites (BOX 2). Some of the commonly used delivery systems are lipid nanoparticles such as neutral lipid emulsions (NLEs) or dendrimer complexes with a targeting moiety attached. Key challenges in translating these delivery systems into the clinic are potential immunostimulatory effects and the lack of specific targeting of the disease site. Once these hurdles are cleared, small RNA therapeutic candidates must undergo rigorous disease-specific *in vivo* testing using rodents and non-human primate models. Careful evaluation of toxicity data and target engagement is required to avoid early failures during clinical trials. DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine; PEI, polyethylenimine; pHLIP, pH low insertion peptide.

mir-155 expression is increased in cancer cells by inflammation-induced signalling molecules such as JNK and nuclear factor-κB (NF-κB)-like transcription factors, which bind to the promoter region of the *mir-155* host gene[83], thereby linking inflammation and cancer. More details on the role of miR-155 and other important miRNAs involved in tumour microenvironment interactions are discussed in BOX 3.

miR-210. One of the most prominent targets of the hypoxia-responsive transcription factor hypoxia-inducible factor 1α (HIF1α) is miR-210, which was identified in an analysis that compared miRNA signatures of cells cultured under hypoxic conditions with cells cultured under normoxia conditions[87]. During the hypoxia response, it was shown that miR-210 targets the mRNA that encodes the mitochondrial electron transport chain component protein succinate dehydrogenase complex subunit D (SDHD). Decreased expression of *SDHD* resulted in an increased stabilization of HIF1α and cancer cell survival[88]. miR-210 also downregulates the hypoxia stress response cell death inducer mitochondrion-associated 3 (AIFM3), thereby promoting survival of cancer cells[89], and ephrin A3, a hypoxia-responsive angiogenesis inhibitor, leading to increased tumour angiogenesis[90]. The cell cycle regulators E2F3 and RAD52 are also downregulated by miR-210, resulting in increased cell cycle G2/M transition and an inefficient DNA damage response, leading to increased DNA instability during cancer growth[91].

miR-17~92 cluster. The miR-17~92 cluster, comprising miR-17, miR-18a, miR-19a, miR-20a, miR-19b and miR-92a, is transcriptionally upregulated in several different malignancies[92,93]. This association was found to be due to the transcriptional upregulation of its host gene *MIR17HG* by MYC[92,93]. The multifaceted roles of the miR-17~92 cluster have been extensively reviewed in REF. 92. For example, this miRNA cluster can downregulate the cell cycle regulator E2F1, thereby counterbalancing the transcriptional activation of E2F1 by MYC and facilitating cell proliferation[93]. The pro-apoptotic protein BIM (also known as BCL2L11) is also downregulated by miR-92a in B lymphocytes, resulting in decreased apoptosis of B lymphocytes[94]. In colon cancer, miR-18a and miR-19a were shown to repress the anti-angiogenic factors thrombospondin 1 (TSP1) and connective tissue growth factor (CTGF)[95]. Together, these results suggest that the miR-17~92 cluster has several different roles in tumour progression and might therefore be an attractive target for miRNA therapeutics. However, loss of the miR-17~92 cluster has been associated with the rare genetic disease Feingold syndrome (presenting with microcephaly, limb structure variations and mental retardation) and with adult-onset deafness[96,97]. Moreover, germline deletion of *Mir17hg* in mouse models resulted in significant increases in microcephaly, short stature and abnormalities in limb development[96].

miR-10b. miR-10b is significantly upregulated in metastatic breast cancer cells compared with non-metastatic or normal breast epithelial cells[98]. Other cancer types such as glioblastoma and melanoma also exhibit upregulated miR-10b[99,100]. TWIST1, a transcription factor involved in increased EMT phenotype of cancer cells, can bind to the *mir-10b* promoter, increasing its expression in breast cancer cells[98]. HOXD10, a member of the homeobox DNA-binding-domain-containing transcription factors, is downregulated by miR-10b, resulting in a pro-metastatic phenotype of breast[101] and ovarian[102] cancers.

miR-221. miR-221 is one of the most significantly upregulated miRNAs in hepatocellular carcinoma (HCC), with mRNA targets including key tumour suppressors such as $p27^{KIP1}$ (also known as CDKN1B), PTEN and tissue inhibitor of metalloproteinases 3 (TIMP3)[103,104]. Conclusive evidence for a causative role of miR-221 in HCC came from a study using a mouse model of HCC, whereby *mir-221* overexpression resulted in increased numbers of tumorigenic murine hepatic progenitor cells[105]. This study identified an additional miR-221 target, DNA damage-inducible transcript 4 (DDIT4), a signalling molecule that is part of the mechanistic target of rapamycin (mTOR) complex.

Therapeutic modulation of miRNAs in cancer

The ability to modulate miRNA expression and activity *in vivo* through miRNA mimics or antimiRs provides an opportunity for the development of innovative therapeutic approaches to cancer. Here, we discuss strategies that are currently in preclinical development to replenish tumour suppressive miRNAs (using miRNA mimics) or to suppress oncomiRs (using antimiRs). Issues in the design of miRNA mimics and antimiRs are discussed in BOX 1, and delivery vehicles for these therapeutics are discussed in BOX 2.

Replenishing tumour suppressive miRNAs

Various strategies have been investigated to replenish miRNAs with tumour suppressive function by using miRNA mimics. Such mimics are synthetically derived oligonucleotide duplexes that mimic the function of a naturally occurring miRNA counterpart. Such miRNA mimics can be modified chemically to have higher stability or to enable the targeted delivery to tumours. These therapeutics can be delivered either systemically or through local injection, and several different delivery vehicles have been investigated (BOX 2).

miR-34. miR-34 mimics, encapsulated in lipid nanoparticles, are the most advanced miRNA therapeutics for cancer and are currently being tested in a phase I clinical trial (NCT01829971) in several solid and haematological malignancies. Several preclinical studies using miR-34 mimics have demonstrated their potential as anticancer therapeutics. For example, lipid nanoparticle-encapsulated miR-34 mimics showed promising activity in mouse models of liver[106], prostate[37] and lung[107] cancer. In models of NSCLC, a liposomal formulation of miR-34 mimics was delivered either locally to xenografted lung tumours or administered systemically to mice with orthotopic lung tumours. In both cases, significant inhibition of tumour growth was

Table 2 | **Selected list of miRNA therapeutics in clinical trials**

Name (company)	Therapeutic agent	Delivery system	Target diseases	Trial details	ClinicalTrials.gov identifier
miRNA-based therapeutics					
Mirvirasen (Santaris Pharma A/S and Hoffmann-La Roche)	AntimiR-122	LNA-modified antisense inhibitor	Hepatitis C (chronic infections included)	Single-centre phase I, completed	NCT01646489
				Multicentre phase II, completed	NCT01200420
				Multicentre phase II, ongoing	NCT01872936
				Single-centre phase II, ongoing	NCT02031133
				Single-centre phase II, ongoing	NCT02508090
RG-101 (Regulus Therapeutics)	AntimiR-122	GalNAc-conjugated antimiR	Chronic hepatitis C	Phase I, completed	–
				Multiple phase II, ongoing	–
RG-125/ AZD4076 (Regulus Therapeutics)	AntimiR-103/107	GalNAc-conjugated antimiR	Patients with type 2 diabetes and non-alcoholic fatty liver diseases	Single-centre phase I, ongoing	NCT02612662
				Single-centre phase I/IIa, ongoing	NCT02826525
MRG-106 (miRagen Therapeutics)	AntimiR-155	LNA-modified antisense inhibitor	Cutaneous T cell lymphoma and mycosis fungoides	Multicentre phase I, ongoing	NCT02580552
MRG-201 (miRagen Therapeutics)	miR-29 mimic	Cholesterol-conjugated miRNA duplex	Scleroderma	Single-centre phase I, ongoing	NCT02603224
MesomiR-1 (EnGeneIC)	miR-16 mimic	EnGeneIC delivery vehicle	Mesothelioma, non-small cell lung cancer	Multi-centre Phase I, ongoing	NCT02369198
MRX34 (Mirna Therapeutics)	miR-34 mimic	LNPs (Smarticles)	Multiple solid tumours	Multicentre phase I, terminated	NCT01829971

DOPC, 1,2 dioleoyl-*sn* glycero-3 phosphatidylcholine; eIF, eukaryotic initiation factor; GalNAc, *N*-acetyl-D-galactosamine; HBV, hepatitis B virus; LNA, locked nucleic acid; LNPs, lipid nanoparticles; miRNA, microRNA; PEI, polyethylenimine; RSV, respiratory syncytial virus.

observed, and tumours expressed lower levels of proteins that are regulated by miR-34, such as MET and BCL-2. Moreover, there was no evidence of adverse effects caused by carrier-mediated immune stimulation.

In an orthotopic model of pancreatic cancer using MiaPaca-2 cells, systemic delivery of miR-34 in liposomal carriers resulted in decreased tumour growth, increased tumour cell apoptosis and decreased $CD44^+$ cell counts, indicating a decrease in metastatic cells[108]. Neutral lipid emulsion-based delivery (BOX 1) of miR-34 in a prostate cancer mouse model achieved only a modest reduction in tumour growth. However, a significant increase in survival times was observed owing to a reduction in metastatic spread to the lung and other tissues[37].

In the ($Kras^{LSL-G12D/+}$;$Trp53^{LSL-R172H/+}$) model of NSCLC, which is highly resistant to anticancer therapies, viral vector-based strategies to deliver inducible miR-34 also showed promise[109]. Upon induction of miR-34 transcription in tumours, a decrease in the levels of the miR-34-regulated anti-apoptotic protein BCL 2 was observed, and the animals experienced a significant reduction in tumour burden.

A subsequent study showed that treatment with an miR-34 mimic (MRX34) encapsulated in lipid nanoparticles that are already approved for human trials in the aggressive *Kras;Trp53* NSCLC mouse model led to significant tumour reduction[106]. Moreover, in this model, a combination approach that enabled co-delivery of let-7 and miR-34 using the same lipid nanoparticle carrier achieved a significant reduction in tumour nodules and extended survival benefit[110]. In addition, combination treatment with the EGFR inhibitor erlotinib and miR-34 and let-7 showed synergistic effects in inhibiting the growth of NSCLC cell lines *in vitro*[110].

miR-200. Another miRNA that has been targeted in preclinical studies is miR-200. In an orthotopic mouse model of lung cancer, systemic treatment of tumours with miR-200c mimics in DOPC (1,2 dioleoyl-*sn* glycero-3 phosphatidylcholine) liposomal carriers resulted in increased radiosensitivity and significantly longer survival compared with controls[111]. The authors demonstrated that in addition to the transcriptional inhibitor ZEB1, miR-200c targets the genes encoding oxidative stress response proteins such as peroxiredoxin 2 (PRDX2), NF-E2-related factor 2 (NRF2; also known as NFE2L2) and sestrin 1 (SESN1). This effect leads to the generation of increased levels of reactive oxygen species (ROS), resulting in cancer cell apoptosis. In a

parallel study, it was demonstrated that miR-200c targets interleukins, and delivery of mimics of miR-200 family members using DOPC lipid nanoparticles in orthotopic mouse models of ovarian (miR-200a/b), basal-like breast (miR-141) and lung (miR-200a/b) cancers resulted in decreased tumour nodules and distant metastasis[61].

miR-26a. In a large panel of RNA samples from patients with HCC ($n = 455$), levels of miR-26a were significantly reduced compared with normal tissues[112]. Furthermore, low levels of miR-26a correlated with poor patient survival[113]. In a murine model of HCC, adeno-associated virus-mediated expression of *mir-26a* resulted in significant tumour regression, which was attributed to the direct targeting of mRNAs encoding the cell cycle controllers cyclin D2 and cyclin E2 (REF. 112).

miR-506 and miR-520. In two separate studies, the delivery of DOPC liposomes containing miR-506 mimics (miR-506 is a regulator of EMT phenotype and the DNA damage response)[64] or mimics of miR-520 (which targets the oncogenes *EPHA2* and *EPHB2*)[68] in ovarian cancer orthotopic mouse models resulted in significant tumour regression and in decreased expression of the respective mRNA targets *in vivo*.

miR-15/16. Ectopic expression of the miR-15/16 cluster using viral vectors in the MEG01 subcutaneous model of leukaemia resulted in a significant reduction in tumour volume and growth[114]. Moreover, delivery of miR-16 using an EGFR-targeted EnGeneIC Delivery Vehicle (EDV) nanocell delivery system (TargomiRs; see BOX 1) in malignant pleural mesothelioma and NSCLC xenograft mouse models resulted in tumour-targeted delivery and significant tumour reduction[115].

Suppressing oncomiRs

Several preclinical studies have investigated anticancer strategies based on the suppression of oncomiRs by using antimiRs based on antisense oligonucleotides (ASOs), locked nucleic acids (LNAs) or antagomiRs (BOX 1).

miR-10b. An early study of the therapeutic potential of antimiRs demonstrated the successful inhibition of miR-10b using ASOs in an orthotopic model of breast cancer[101]. This antimiR resulted in decreased metastasis due to rescue of the expression of the anti-metastatic gene *HOXD10*. However, the authors observed no reduction in primary tumour growth, suggesting the need for initial tumour reduction surgery or chemotherapy combinations. It will be interesting to determine whether such miR-10b inhibitors affect long-term survival.

Interestingly, in an orthotopic glioblastoma mouse model, delivery of an antagomiR (BOX 1) against miR-10b using *in vivo*-jetPEI, a commercially available PEI delivery system, resulted in a significant reduction in tumour growth[116]. This result needs further evaluation considering that HOXD10 is not thought to play a part in the growth of the primary tumour, and suggests a role of miR-10b beyond downregulating HOXD10.

A recent study combined LNAs against miR-10b with doxorubicin in mouse models of breast cancer[117]. Delivery of miR-10b LNAs encapsulated in a thiomagnetic nanocarrier enabled imaging of the nanoparticles using near-infrared imaging. A combination of a low dose of doxorubicin with miR-10 ASOs achieved a significantly greater decrease in tumour burden compared with doxorubicin monotherapy. Furthermore, no evidence of damage to normal tissue was observed, suggesting that there was no toxicity associated with the delivery of this LNA nanoparticle.

miR-221. miR-221 is one of the most significantly upregulated miRNAs in HCC, in which miR-221 downregulates key tumour suppressors such as $p27^{KIP1}$, PTEN and TIMP3 (REFS 103,104). A cholesterol-modified form of antimiR-221, delivered intravenously to HCC xenografts, showed significant activity in downregulating miR-221 and increasing the levels of its mRNA targets[118]. Mice treated with antimiR-221 experienced tumour shrinkage and survived significantly longer than control mice. However, the lack of rigorous toxicity data currently limits the use of this cholesterol-modified antimiR for further development.

miR-155. Using a mouse model of miR-155-induced lymphoma, in which *mir-155* expression is under the control of doxycycline (*mir-155*^{LSLrTA}), it was demonstrated that doxycycline withdrawal resulted in the shutdown of *mir-155* expression and subsequent tumour shrinkage beyond detection limits[85]. In the same mouse model, delivery of antimiR-155, packaged in poly(lactic-co-glycolic acid) nanoparticles (BOX 1), resulted in a decreased tumour burden, indicating that inhibition of miR-155 might have therapeutic potential[85]. Recently, a pH-sensitive antimiR-155 conjugate called pHLIP–antimiR-155 was tested in this model[119]. pHLIP (pH low insertion peptide) is a small peptide that forms a transmembrane α-helix under acidic conditions[120]. Because the tumour microenvironment is acidic, a conjugate of pHLIP and antimiR-155 facilitated the specific delivery of antimiR to cancer cells (FIG. 2). Mice treated with pHLIP–antimiR-155 exhibited a significant reduction in tumour burden, resulting in prolonged survival. No significant toxicity was observed, suggesting that the clinical translation of this approach may be feasible[119].

miR-630. miR-630 is an oncomiR that is upregulated in response to hypoxia in the tumour environment. Using an antimiR against miR-630 and the DOPC delivery platform in an orthotopic model of ovarian cancer, a significant reduction in tumour growth and metastasis was observed[33].

miRNAs in diseases other than cancer

As miRNAs are important for various cellular homeostasis functions, their role extends to a number of disease manifestations beyond cancer. *In vivo* delivery of miRNA mimics or inhibitors has been successfully achieved in mouse models of hepatitis, cardiac diseases and diabetes-associated kidney fibrosis (TABLES 1,2).

Box 3 | **miRNA alterations in the tumour microenvironment**

Cells in the tumour microenvironment, such as endothelial cells, fibroblasts and immune cells, interact with the cancer cells by secreting factors that modulate tumour microenvironment physiology, including hypoxia, pH and inflammation[10]. MicroRNAs (miRNAs) regulate these interactions by targeting several genes involved in these interactions (such as the genes encoding nuclear factor-κB (NF-κB) and SHIP1, which are involved in inflammatory responses) and act as pro-tumoural signals. Targeting these miRNAs for therapy needs to be approached carefully because the role of such miRNAs can be highly context dependent. For example, it was long thought that the miR143/145 cluster is a tumour suppressor in cancer cells; however, a recent study showed miR-143/145 can induce neoangiogenesis in the tumour microenvironment, leading to increased tumour growth[185]. The role of miRNAs in the tumour microenvironment has been discussed in several reviews[10,186,187]. Here, we highlight selected prominent examples of miRNAs that are involved in the tumour microenvironment.

miRNAs and cancer-associated fibroblasts

Cancer-associated fibroblasts (CAFs) provide a stromal framework for the cancer cells to adhere and grow during initial malignancy and metastasis processes. One of the important miRNAs known to play a part in transforming normal fibroblasts to CAFs is miR-320 (REF. 188). Downregulation of miR-320 in normal fibroblasts results in an increase in the mRNA target ETS2, a cancer-specific transcription factor, resulting in increased oncogenic secretome-containing proteinases such as matrix metalloproteinases (MMPs). Although not tested, the replacement of miR-320 in fibroblasts could have a beneficial anti-metastatic role.

miRNAs and inflammation

Inflammation in the tumour microenvironment generally has a pro-tumorigenic role by altering fibroblasts phenotype, resulting in enhanced angiogenesis (by CAF-secreted angiokines such as C-X-C motif ligand (CXCL) chemokines) and invasion of cancer cells (by CAF-secreted proteinases such as MMPs). miRNAs can significantly increase the expression of inflammation-related proteins such as NF-κB, resulting in pro-tumoural changes in the microenvironment. *LIN28*–let-7-mediated derepression of the cytokine interleukin-6 cascade results in activation of NF-κB in cancer cells, which results in a further increase in inflammatory signals[51]. Disruption of this positive feedback loop in the tumour microenvironment via the delivery of let-7 mimics can lead to a drastic effect. *let-7* expression in cancer cells reduces proliferation and enhances apoptosis, and when expressed in cells in the tumour microenvironment, it can reduce inflammation, leading to a less conducive environment for the tumour growth[51].

miR-155 is key oncogenic miRNA because it acts in cancer cells and in tumour microenvironment-associated cells. miR-155 targets SHIP1, a protein involved in the modulation of immune responses[81]. WEE1, a checkpoint kinase involved in the DNA damage response, cell cycle progression during inflammation and cancer development, is downregulated by increased miR-155 levels in cancer cells[84]. Studies have shown that increased *mir-155* expression in normal fibroblasts resulted in conversion of normal fibroblasts into CAFs[189]. Moreover, in preclinical models, treatment with pH-induced transmembrane localization peptide conjugated antimiR-155 resulted in significant tumour reduction[85,119].

In summary, before targeting miRNAs that are known to have a pro-tumoural function in cancer cells, one needs to first carefully assess their role in cells of the tumour microenvironment. Testing the effect of such miRNAs in a comprehensive manner will provide information regarding the context-dependent functions of miRNAs, and will facilitate the identification of suitable targets for cancer therapy.

miR-122 and hepatitis C infection

In contrast to the widely accepted mechanism of mRNA silencing due to miRNA binding, miR-122 upregulates the replication of the hepatitis C virus (HCV) RNA genome[121]. Complementary sites to miR-122 in the 3′ and 5′ end of the non-coding region (NCR) of the HCV viral RNA were reported to have a key role in promoting viral RNA stability[121]. Using deletion and mutation studies, the authors demonstrated the importance of miR-122 binding at the 5′ end of the NCR for the accumulation of the virus, leading to an increased infection rate. Recently, it was shown that miR-122 binding acts as a cap for the 5′ end of the NCR, resulting in protection of viral RNA from the degradation pathway involving the Xrn1 exoribonuclease[122].

In addition to increasing the stability of the HCV RNA, miR-122 binding to the viral RNA results in a 'sponge effect', in which free extracellular or intracellular miR-122 is sequestered at the infection site, leading to a decreased overall abundance of miR-122 (REF. 123). This reduction in miR-122 level affects liver homeostasis, which can lead to liver damage and increases the risk of developing HCC[123]. Inhibition of miR-122 using LNAs resulted in a significant reduction in infection load and reduced liver damage in mouse models of HCV infection[124,125]. Moreover, the authors identified that miR-122 has several target mRNAs that encode proteins involved in the development of HCC; such proteins include prolyl 4-hydroxylase subunit α1 (P4HA1), pyruvate kinase PKM and mannan-binding lectin serine protease 1 (MASP1). Considering the high viral titre during HCV infection, inhibition of miR-122 may serve as an attractive target for improved therapeutic management of the infection; however, a careful assessment needs to be performed regarding the effect of inhibiting miR-122 in host cells such as hepatocytes, because *mir-122*-knockout mice develop liver cancer[126,127].

The first report of using miR-122-targeted LNAs to treat HCV infection demonstrated reduced viral titres in mice[124] and in non-human primates[125]. Subsequently, LNAs against miR-122 achieved a significant reduction in viral titres in clinical trials of HCV-infected patients (TABLE 2). In preclinical studies, a 15-nucleotide phosphorothioate DNA–LNA mixmer called SPC3649 (currently in clinic trials as miravirsen by Santaris Pharma-Denmark,

now a subsidiary of Roche) achieved significantly higher binding affinity to RNA targets and better cellular uptake compared with other cholesterol-based[128] antimiR conjugates[124]. In addition, the authors showed a significant reduction (>300-fold) in virus titres, and no sign of a rebound in viral titres was observed after discontinuation of treatments. Currently, there are two companies (Roche/Santaris and Regulus Therapeutics) engaged in clinical trials using antimiR-122 LNAs as a therapy against HCV infections.

Cardiovascular disease

Cardiovascular diseases have a high mortality rate. Several miRNAs have key roles in different aspects of the progression of cardiovascular diseases such as cardiac hypertrophy and fibrosis and myocardial infarction[11,21]. For example, miR-21 is significantly upregulated during fibrosis of myocytes and causes cardiac hypertrophy, a condition resulting from the gradual loss of myocytes and systemic hypertension[21,129]. SPRY1, a ERK–MAPK pathway molecule, is a direct target of miR-21, and its expression was rescued upon antimiR-21 treatment in a mouse model[130].

In the failing myocardium, a significant increase in *mir-21* expression occurs compared with normal myocardium[130]. Knockdown of *SPRY1* in cardiac fibroblasts correlated with a significant increase in fibrosis and apoptosis, similar to what is observed following treatment with miR-21 mimics, indicating a causal role of miR-21-mediated SPRY1 downregulation in the failing myocardium[130]. As mentioned above, other important targets of miR-21 include PTEN and PDCD4, which are involved in cell survival and the inflammatory response[76].

During kidney injury, miR-21 causes deleterious effects by altering metabolic pathways, which leads to increased fibrosis[131]. miR-21 directly targets the expression of peroxisome proliferator-activated receptor-α (PPARα) during kidney injury, resulting in defective ROS inhibition. PPARα regulates ROS signalling in a negative feedback loop, whereby a disruption of this loop by direct targeting of PPARα by miR-21 results in an accumulation of ROS, leading to renal cell apoptosis. Delivery of an antimiR-21 construct with phosphorothioate backbone modifications to a mouse model of kidney injury resulted in reduced ROS accumulation in the kidney and reduced damage to epithelial cells[131].

Another miRNA family that is involved in heart disease is the miR-143/145 cluster, which is abundantly expressed in vascular smooth muscle cells (VSMCs)[132,133]. miR-143/145 targets several mRNAs that encode proteins involved in the proliferation and differentiation of VSMCs. Such proteins include ETS1, Krueppel-like factor 4 (KLF4) and KLF5, actin-remodelling proteins such as slingshot 2 (SSH2), SLIT-ROBO Rho GTPase-activating protein 1 (SRGAP1) and SRGAP2, and the contractility mediator ACE[134,135]. Downregulation of the miR-143/145 family in mouse models results in hypertension and cardiac failure[132–135].

miR-1, an abundant miRNA in normal heart muscle cells, is decreased in cardiac hypertrophy and fibrosis. miR-1 controls calcium signalling in heart muscles by targeting the genes encoding the calcium-binding protein calmodulin and the transcription factor myocyte enhancer factor 2 (MEF2A)[136]. An important direct target of miR-1 is the growth factor insulin-like growth factor 1 (IGF1), which is involved in the proliferation and survival of the majority of cells in the body. Loss of miR-1 leads to an upregulation of IGF1 secretion, which causes in increased heart mass and wall thickness and subsequent decreased heart function[137].

miR-208 is a cardiac-specific miRNA that is transcribed from the gene that also encodes α-myosin heavy chain (αMHC). miR-208 has a negative role during cardiac stress through the direct targeting of thyroid hormone receptor-associated protein 1 (THRAP1; also known as MED13), a component of the mediator complex[138], which controls cardiac energy homeostasis by regulating thyroid hormone receptors. In the Dahl hypertensive rat model, delivery of antimiR-208 LNAs resulted in significant improvement in cardiac function by reversing myosin switching (a process observed in cardiac hypertrophy involving the change of αMHC to βMHC) during cardiac failure[139].

In a mouse model of bleomycin-induced pulmonary fibrosis, delivery of an antagomiR against miR-29 resulted in a significant reversal of fibrosis[140]. miR-29 accumulated in lung tissues, with a concomitant downregulation of the miR-29 targets collagen α1(I) chain (COL1A1) and collagen α1(III) chain (COL3A1), both of which are matrix proteins and their expression is often increased during fibrosis. Given the role of miR-29 in systemic fibrosis, delivery of miR-29 mimics may provide a therapeutic approach for treating fibrosis.

Atherosclerosis

Atherosclerosis, a disease caused by the build-up of fatty plaques in the inner wall of blood vessels, results in significant risk of stroke and death. During disease manifestation, a significant reduction in the expression of genes that are involved in cellular cholesterol export (ATP-binding cassette subfamily A member 1 (*ABCA1*)), fatty acid oxidation (carnitine *O*-octanoyltransferase (*CROT*) and carnitine palmitoyltransferase 1A (*CPT1A*)), insulin signalling and glucose production (5′-AMP-activated protein kinase (*AMPK*), phosphoenolpyruvate carboxykinase 1 (*PCK1*) and glucose-6-phosphatase catalytic subunit (*G6PC*)) is observed. The expression of these genes is downregulated by miR-33 (REFS 141,142). In non-human primates fed a high-fat diet, treatment with antimiR-33 resulted in increased levels of transcription of miR-33 mRNA targets (mainly the cholesterol transporter ABCA1 and those involved in fatty acid oxidation, such as CROT and HADHB)[143]. This study utilized a 2′-fluoro, 2′-*O*-methoxyethyl phosphorothioate backbone-modified antimiR-33 (Regulus Therapeutics) and reported no adverse effects[143]. However, it should be taken into consideration that these effects are based on 10 weeks of treatment with antimiR-33. A recent study based on longer-term treatment (20 weeks) with antimiR-33 in mice showed circulating triglyceride levels and lipid accumulation in the liver, leading to hepatic steatosis[144]. This effect was attributed to

miR-33 directly targeting fatty acid synthesis-related genes such as nuclear transcription factor Y subunit gamma (*NFYC*; which leads to SREBP transcriptional activation), acetyl-CoA carboxylase (*ACC*) and *FAS* in the liver. This observation provides an example of the type of safety issues that need to be considered before moving any miRNA-targeted approaches into the clinic. As miRNAs target multiple mRNAs, it is crucial to monitor long-term effects in preclinical studies. However, one must note that there are two isoforms (miR-33a and miR-33b) in humans and monkeys compared with a single isoform in mice. The differences that were observed between the mouse and monkey studies might be attributed to the variations in the levels of the different isoforms, wherein each isoform targets a slightly different set of mRNAs.

Diabetes

Several miRNAs are involved in the development of diabetic complications by targeting key genes involved in inflammation, cholesterol metabolism and glucose metabolism. A major manifestation of diabetes is decreased insulin production due to pancreatic β-cell dysfunction and reduced insulin action in peripheral tissues. miR-200a targets the genes encoding the caspase inhibitor X-linked inhibitor of apoptosis protein (XIAP) and β-cell chaperone p58^{IPK}. miR-200a-mediated downregulation of these proteins leads to the apoptosis of β-cells and thereby to decreased insulin production[145]. Downregulation of miR-200 during retinopathy manifestations, a complication most commonly seen in patients with diabetes, results in increased neovascularization owing to the increased production of pro-angiogenic VEGF proteins. Loss of miR-200b has been attributed to these phenotypes due to derepression of VEGF expression[146]. Use of miR-200 mimics in this setting provides an attractive therapeutic strategy for the clinical management of this disease.

miR-192 targets E-cadherin, a key regulator of epithelial cell morphology. Downregulation of E-cadherin leads to fibrosis of tubular cells, thereby causing diabetic nephropathy[147]. In the apolipoprotein E mouse model of diabetes, a decrease in miR-192 and an increase in transcription factor *Zeb2* expression was observed in the kidneys. Using ectopic expression of *mir-192*, the authors demonstrated that decreased expression of ZEB2 can result in increased E-cadherin expression owing to the loss of ZEB2-mediated transcriptional repression[147]. These findings indicate that miR-192 mimics might potentially be of use as therapeutics, and preclinical studies are pending.

Three members of the miR-29 family, miR-29a, miR-29b1 and miR-29b2, are also closely associated with the development of diabetes. An increase in these three miRNAs was observed in the liver, kidney, pancreatic β-cells and in adipose tissues in patients with diabetes[148]. The anti-apoptotic protein MCL1 is one of the crucial targets of miR-29 family members, and increased levels of miR-29a, miR-29b and miR-29c in these tissues results in cellular apoptosis, thereby promoting inflammation and tissue damage[149].

Scleroderma

In patients with systemic sclerosis (scleroderma), a chronic connective tissue disease involving fibrosis, miR-29 is significantly decreased in fibroblasts, resulting in fibrosis due to increased expression of the collagens COL1A1 and COL3A1, which are normally downregulated by miR-29 (REF. 150).

Clinical studies involving miRNAs

In the short time since the discovery of miRNAs, therapeutic approaches to manipulate them have progressed from bench to bedside, with some successful phase I trials and ongoing phase II trials.

One of the first miRNA-based molecules to enter clinical development was the LNA miravirsen, a 15-nucleotide antisense RNA oligo with complementarity to the 5′ end of miR-122, for the treatment of HCV. This treatment takes advantage of the natural accumulation of systemically delivered miRNA in the liver. Based on preclinical studies in rodents and in non-human primates that showed efficacious delivery to the liver, reduced cholesterol accumulation (suggesting improved liver function) and reduced titres of HCV, a phase I clinical trial was initiated in 2009 (REFS 22,124,125). Consistent with non-human primate study results, no adverse reactions were observed, encouraging the launch of a phase IIa trial[151]. In the first phase IIa trial, a total of 36 patients were enrolled into 4 groups (9 patients per group), receiving a dose of 3 mg, 5 mg or 7 mg per kg miravirsen or placebo once per week for 5 weeks. Patients in this study experienced a significant dose-dependent reduction in HCV load[151]. Viral rebound was observed in a small set of patients receiving miravirsen (in 1 patient receiving 3 mg per kg, 5 patients receiving 5 mg per kg, and 3 patients receiving 7 mg per kg), and no miRNA target site mutations were observed. Interestingly, out of 112 adverse events reported, the majority were grade one (such as headache) and only 1 patient had a grade 3 adverse event (thrombocytopaenia). During an 18-week follow-up period, 5 patients in the miravirsen and 2 patients in the placebo groups, respectively, had grade 1 adverse events (such as headache) with clinically manageable severity, suggesting that the treatment is safe. Furthermore, decreased serum cholesterol was observed, which could be a biomarker for treatment efficacy[151]. The success of these phase I and II trials has led to the advancement of this drug into additional phase II studies with long-term follow-ups, more patients and multidrug combinations (TABLE 2).

A recent report showed mutations near the end of 5′ UTR of HCV viral RNA in both *in vitro* samples treated with increasing dose of miravirsen and in clinical samples from the patients receiving miravirsen[152]. In the samples from *in vitro* experiments, the observed mutation was A4C (after 148 days of culture, with 80 μM miravirsen). Interestingly, in clinical samples, upon sequencing the 5′ end UTR, the mutation observed was C3A (5 out of 6 patients, during 18 weeks of follow-up). Even though these mutations occurred in miravirsen-treated cells or patients with viral rebound, it is not clear whether these mutations can cause resistance to therapy.

This phenomenon will require a deeper analysis of miravirsen binding sites and a cause versus correlation analysis using suitable *in vitro* and *in vivo* techniques.

RG-101 (Regulus Therapeutics), a *N*-acetyl-D-galactosamine (GalNAc)-conjugated antimiR against miR-122, has also undergone phase I trials in HCV-infected patients. A dose of 2–4 mg per kg was effective as a single dose, achieving a significant reduction in viral load (see Regulus Therapeutics, press release dated 9 February 2015). Data on viral rebound during long-term monitoring of the patients showed HCV levels below quantification range. A phase II trial that combines RG-101 with direct-acting antivirals such as Harvoni (a combination of ledipasvir and sofosbuvir) for prolonged effects of therapy is currently in progress (TABLE 2). Interim data show a 100% response rate with the combination of miR-122 and Harvoni, and showed no relapse at 24 weeks (see Regulus Therapeutics, press release dated 7 June 2016). However, the trial was put on hold by the US FDA after a second case of jaundice was reported (see Regulus Therapeutics, press release dated 1st Nov 2016).

With regard to the development of cancer therapeutics based on miRNA mimics, the most advanced compound is MRX34, a miR-34 mimic (Mirna Therapeutics) encapsulated in a lipid carrier called NOV40 (REF. 19). NOV40 particles have the advantage of becoming positively charged under low pH conditions such as in the tumour microenvironment, which allows them to adhere to tumour cells[111]. In mice treated with MRX34 nanoparticles, an accumulation of miR-34 in tumours was observed, as well as significant tumour regression[19,44,47,110]. MRX34 entered a multicentre phase I trial in 2013 in patients with primary liver cancer, small cell lung cancer, lymphoma, melanoma, multiple myeloma or renal cell carcinoma. The trial included a dose-escalation study with a two times per week or five times per day schedule, with MRX34 being administered via intravenous infusion. As of June 2016 (REF. 153), a total of 99 patients with HCC, NSCLC or pancreatic cancer had been enrolled in the study[154]. At the end of the trial, 3 patients (1 with HCC, 1 with renal cell carcinoma and 1 with acral melanoma) achieved prolonged confirmed partial responses as per RECIST (response evaluation criteria in solid tumours) and 14 patients had stable disease (median duration 136 days; range 79–386 days). Analysis of white blood cell samples showed significant reduction in the miR-34 target mRNAs such as forkhead box P1 (*FOXP1*) and *BCL2*. However, owing to immune-related adverse events involving patient deaths, the trial was terminated (see Mirna Therapeutics Halts Phase 1 Clinical Study of MRX34). As the cause of these immune reactions is unclear, pre-clinical trials will need to be re-designed, with a special emphasis on the study of immune-related toxicities.

In a collaboration between EnGeneIC and the Asbestos Diseases Research Institute, Sydney, Australia, miR-16 therapeutics using mimics have entered phase I trials in patients with malignant pleural mesothelioma or NSCLC. In a first open-label safety and efficacy study, miR-16 was delivered in an EDV nanocell with EGFR antibody surface conjugation, which facilitates targeting to the tumour site[115,155]. Preliminary data reported manageable safety in response to infusion of 5 billion nanocells loaded with 1.5 µg miR-15/16 mimics as a first dose level in the first five patients that had been enrolled[155].

Recently, two phase I clinical trials of the miR-29 miRNA mimic MRG-201 (miRagen Therapeutics) in patients with scleroderma and the LNA-based antimiR-155 (MRG-106; miRagen Therapeutics) in patients with cutaneous T cell lymphoma, mycosis fungoides subtype were initiated. Interim reports from both of the studies are expected in early 2017.

Non-alcoholic steatohepatitis (NASH, a non-alcoholic fatty liver disease) is often diagnosed in patients with obesity, dyslipidaemia and glucose intolerance due to reduced insulin sensitivity during diabetes manifestations. RG-125, a GalNAc-conjugated antimiR against miR-103/107, recently entered clinical investigations for the treatment of NASH. *mir-103/107* expression is increased in the liver in obese mice (leptin-deficient (*ob*/*ob*) and diet-induced obese mice) and in the liver of patients with NASH[156]. Caveolin 1, a protein involved in insulin signalling and sensitivity, was shown to be the direct mRNA target of miR-103/107. In preclinical studies, delivery of an antagomiR against miR-103/107 in a mouse model of obesity with insulin resistance resulted in significantly increased insulin sensitivity[156].

Challenges and future directions

Although a considerable number of preclinical studies involving miRNA therapeutics have been conducted over the years, only a small number of miRNA therapeutics have so far moved into clinical development. One of the biggest challenges in developing miRNA-based therapeutics is to identify the best miRNA candidates or miRNA targets for each disease type. Other challenges include the design of miRNA delivery vehicles that confer higher stability to the therapeutic candidate and enable tissue-specific targeting, as well as avoiding potential toxicities and off-target effects[20,157,158].

One key obstacle in identifying the relevant target miRNA is posed by the significant heterogeneity of miRNA expression. For example, in cancer, factors such as hypoxia and inflammation in the tumour microenvironment cause complex and dynamic regional heterogeneity, complicating the identification of candidate miRNAs[6,9,10,33,34,159,160]. Moreover, key miRNA biogenesis enzymes such as Drosha, Dicer and AGO2 are downregulated by hypoxia, leading to aberrant miRNA expression[33–35,160]. Biopsy samples only probe one specific area and do not provide insight into the dynamics of miRNA expression. To overcome the obstacles posed by the heterogeneity of miRNA expression in cancer, biopsy samples of various tissues (ideally multiple biopsies taken at different times) during disease progression would be needed to identify common regulatory miRNAs, which can then be therapeutically manipulated.

The advent of genomics and new sequencing approaches have led to a huge increase in data pertaining to disease manifestations, often with the same type of data available from multiple tissue resources. For example, several hundred data repositories exist that profile cancer cell lines or tissue samples of cancers at various

stages of progression. A systematic analysis of these data sets to understand miRNA-target networks might enable us to identify key common miRNAs involved in disease processes. For example, a recent integrative analysis of miRNA regulatory networks identified miR-506 as a central miRNA that regulates different aspects of the metastatic process in ovarian cancer[64]. Using miRNA and mRNA data from the public repository of cancer genomic and proteomic data (TCGA), the authors investigated miRNA and mRNA networks in ovarian cancer cells of the mesenchymal subtype. This 'targetome' bioinformatic analysis of miRNA–mRNA interactions and inverse expression patterns of particular miRNAs and mRNAs revealed that miR-506, miR-101, miR-200a, miR-25, miR-128 and miR-182 are significantly downregulated in the mesenchymal subtype of ovarian cancer. They further showed that miR-506 becomes undetectable during EMT, and that restoring miR-506 in ovarian cancer cells results in a reversal of cell phenotype from the mesenchymal to the epithelial type; this finding was detected using *in vitro* and *in vivo* experiments. Interestingly, patients expressing high levels of *mir-506* had experienced significantly longer progression-free survival, and preclinical experiments showed tumour regression in response to the delivery of miR-506 mimics via the liposomal carrier DOPC[64].

The availability of databases such as TCGA has helped researchers worldwide to discover novel miRNA pathways involved in cancer. More precise identification of the miRNA targetome was enabled by recent techniques such as miR-CLIP seq. Using this technique, miRNA–mRNA associations can be identified through biochemical pull-down assays of specific miRNA and associated mRNA targets, followed by sequencing.

A recent study showed that the miR-CLIP capture technique can also identify novel interactions between miRNAs and long non-coding RNAs (lncRNAs), another class of non-coding RNAs that are often aberrantly expressed in diseases[161]. The authors performed a miR-CLIP analysis with miR-106a and found that lncRNA H19 has several binding sites for miR-106a. It was shown that lncRNA H19 has a sponging effect on miR-106a, effectively titrating the miRNA away from its mRNA targets and thereby upregulating the level of the respective mRNAs. Given the recent advances in our understanding of the roles of lncRNAs in cancer and other diseases, it will be highly interesting to learn how lncRNAs and miRNAs interact with each other in normal cell homeostasis and diseases.

There are currently several databases that have a repository of similar CLIP-seq data involving miRNAs. For example, the recently updated miRTarBase[162] or starBase[163] have incorporated these publicly available data on CLIP-seq into a single platform for comprehensive analyses. Combining these databases, along with prediction tools such as TargetScan[164], will help researchers to more accurately predict mRNA targets of miRNAs and enable a more efficient identification of the disease-relevant miRNAs and mRNAs.

Another strategy to find key regulatory miRNAs in disease-specific processes is to carry out genome-wide functional screens using miRNA mimics or inhibitors. For example, a high-content screen for proliferation-related miRNAs in neonatal rat cardiomyocytes using a library of 875 miRNA mimics identified miR-590 and miR-199a as key miRNAs involved in neonatal cardiomyocyte regeneration[165]. Systemic delivery of these miRNAs using lipid carriers or adeno-associated virus-mediated delivery to neonatal rats resulted in increased numbers of cardiomyocytes and a reduction in fibrosis[165].

Another approach that has not yet been extensively explored is the identification of miRNAs that resensitize chemoresistant cancer cells. *In vitro* and *in vivo* studies have shown that miRNAs such as let-7, miR-34, miR-451 and miR-200 can sensitize cancer cells to chemotherapy[110,166,167], and mimics of these miRNAs could be rationally combined with chemotherapeutic drugs.

A clear picture of the miRNA targetome, defining the number of oncogenes or tumour suppressors targeted by a particular miRNA, has yet to be drawn. The ability of miRNAs to target multiple genes is attractive, as this feature may facilitate the targeting of multiple compensatory pathways. However, a particular miRNA targetome might include both oncogenes and tumour suppressors, as well as a number of targets not involved in cancer, which complicates the development of selective miRNA-directed therapeutics. Moreover, miRNAs, especially at non-physiological concentrations, can have unknown targets that could potentially lead to adverse effects by targeting normal cell homeostasis genes. Thus, it is essential to carefully and comprehensively investigate the mRNA 'targetome' of a particular miRNA before proceeding to therapeutics. Finally, the question of whether individual patients (intertumoural heterogeneity) express different sets of driver miRNAs remains to be tested.

In summary, several preclinical formulations have shown promise with a low toxicity profile and with their payload delivered to the target site. In our opinion, the current surge in genomic and proteomic data in human biology will aid in the identification of key miRNA targets for drug development. This increased body of knowledge, coupled with a comprehensive preclinical analysis aided by novel delivery platforms, should enable miRNA therapeutics to become a long-term clinical reality.

1. Bartel, D. P. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **116**, 281–297 (2004).
2. Lee, R. C., Feinbaum, R. L. & Ambros, V. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* **75**, 843–854 (1993).
Discovery of the first miRNA, lin-4, and elucidation of its function in development.
3. Reinhart, B. J. *et al.* The 21-nucleotide *let-7* RNA regulates developmental timing in *Caenorhabditis elegans*. *Nature* **403**, 901–906 (2000).
Discovery of the second miRNA, let-7, and description of its role of in the development of *C. elegans*.
4. Pasquinelli, A. E. *et al.* Conservation of the sequence and temporal expression of *let-7* heterochronic regulatory RNA. *Nature* **408**, 86–89 (2000).
5. Esteller, M. Non-coding RNAs in human disease. *Nat. Rev. Genet.* **12**, 861–874 (2011).
6. Coussens, L. M. & Werb, Z. Inflammation and cancer. *Nature* **420**, 860–867 (2002).
7. Roush, S. & Slack, F. J. The *let-7* family of microRNAs. *Trends Cell Biol.* **18**, 505–516 (2008).
8. Iorio, M. V. & Croce, C. M. MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. *EMBO Mol. Med.* **4**, 143–159 (2012).
9. Tili, E., Michaille, J. J. & Croce, C. M. MicroRNAs play a central role in molecular dysfunctions linking inflammation with cancer. *Immunol. Rev.* **253**, 167–184 (2013).

10. Rupaimoole, R., Calin, G. A., Lopez-Berestein, G. & Sood, A. K. miRNA deregulation in cancer cells and the tumor microenvironment. *Cancer Discov.* **6**, 235–246 (2016).
11. Gurha, P. MicroRNAs in cardiovascular disease. *Curr. Opin. Cardiol.* **31**, 249–254 (2016).
12. Worringer, K. A. *et al.* The let-7/LIN-41 pathway regulates reprogramming to human induced pluripotent stem cells by controlling expression of prodifferentiation genes. *Cell Stem Cell* **14**, 40–52 (2014).
13. Ha, M. & Kim, V. N. Regulation of microRNA biogenesis. *Nat. Rev. Mol. Cell Biol.* **15**, 509–524 (2014).
14. Lin, S. & Gregory, R. I. MicroRNA biogenesis pathways in cancer. *Nat. Rev. Cancer* **15**, 321–333 (2015).
15. Hill, D. A. *et al. DICER1* mutations in familial pleuropulmonary blastoma. *Science* **325**, 965 (2009).
16. Heravi-Moussavi, A. *et al.* Recurrent somatic *DICER1* mutations in nonepithelial ovarian cancers. *N. Engl. J. Med.* **366**, 234–242 (2012).
Evaluates mutations in the miRNA biogenesis enzyme Dicer in cancers using patient tumour samples.
17. Rakheja, D. *et al.* Somatic mutations in *DROSHA* and *DICER1* impair microRNA biogenesis through distinct mechanisms in Wilms tumours. *Nat. Commun.* **2**, 4802 (2014).
18. Melo, S. A. *et al.* A genetic defect in exportin-5 traps precursor microRNAs in the nucleus of cancer cells. *Cancer Cell* **18**, 303–315 (2010).
Identifies mutations in the exportin 5 enzyme in cancer cells.
19. Bader, A. G. miR-34 — a microRNA replacement therapy is headed to the clinic. *Front. Genet.* **3**, 120 (2012).
20. Li, Z. & Rana, T. M. Therapeutic targeting of microRNAs: current status and future challenges. *Nat. Rev. Drug Discov.* **13**, 622–638 (2014).
21. van Rooij, E. & Olson, E. N. MicroRNA therapeutics for cardiovascular disease: opportunities and obstacles. *Nat. Rev. Drug Discov.* **11**, 860–872 (2012).
22. van Rooij, E. & Kauppinen, S. Development of microRNA therapeutics is coming of age. *EMBO Mol. Med.* **6**, 851–864 (2014).
23. Karube, Y. *et al.* Reduced expression of Dicer associated with poor prognosis in lung cancer patients. *Cancer Sci.* **96**, 111–115 (2005).
24. Merritt, W. M. *et al.* Dicer, Drosha, and outcomes in patients with ovarian cancer. *N. Engl. J. Med.* **359**, 2641–2650 (2008).
A large study utilizing tumour samples to evaluate miRNA biogenesis enzyme Dicer and Drosha deregulations in cancer and the relationship to patient outcomes.
25. Wang, X., Zhao, X., Gao, P. & Wu, M. c-Myc modulates microRNA processing via the transcriptional regulation of Drosha. *Sci. Rep.* **3**, 1942 (2013).
26. Allegra, D. *et al.* Defective DROSHA processing contributes to downregulation of MiR-15/-16 in chronic lymphocytic leukemia. *Leukemia* **28**, 98–107 (2014).
27. Torres, A. *et al.* Major regulators of microRNAs biogenesis Dicer and Drosha are down-regulated in endometrial cancer. *Tumour Biol.* **32**, 769–776 (2011).
28. Dedes, K. J. *et al.* Down-regulation of the miRNA master regulators Drosha and Dicer is associated with specific subgroups of breast cancer. *Eur. J. Cancer* **47**, 138–150 (2011).
29. Guo, X. *et al.* The microRNA-processing enzymes: Drosha and Dicer can predict prognosis of nasopharyngeal carcinoma. *J. Cancer Res. Clin. Oncol.* **138**, 49–56 (2012).
30. Su, X. *et al. TAp63* suppresses metastasis through coordinate regulation of Dicer and miRNAs. *Nature* **467**, 986–990 (2010).
Documents the transcriptional regulation of Dicer in cancer.
31. Martello, G. *et al.* A microRNA targeting dicer for metastasis control. *Cell* **141**, 1195–1207 (2010).
32. Tokumaru, S., Suzuki, M., Yamada, H., Nagino, M. & Takahashi, T. *let-7* regulates Dicer expression and constitutes a negative feedback loop. *Carcinogenesis* **29**, 2073–2077 (2008).
33. Rupaimoole, R. *et al.* Hypoxia-upregulated microRNA-630 targets Dicer, leading to increased tumor progression. *Oncogene* **35**, 4312–4320 (2016).
34. van den Beucken, T. *et al.* Hypoxia promotes stem cell phenotypes and poor prognosis through epigenetic regulation of DICER. *Nat. Commun.* **5**, 5203 (2014).
35. Shen, J. *et al.* EGFR modulates microRNA maturation in response to hypoxia through phosphorylation of AGO2. *Nature* **497**, 383–387 (2013).
36. Zhang, X., Wan, G., Berger, F. G., He, X. & Lu, X. The ATM kinase induces microRNA biogenesis in the DNA damage response. *Mol. Cell* **41**, 371–383 (2011).
37. Liu, C. *et al.* The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. *Nat. Med.* **17**, 211–215 (2011).
38. Rokavec, M. *et al.* IL-6R/STAT3/miR-34a feedback loop promotes EMT-mediated colorectal cancer invasion and metastasis. *J. Clin. Invest.* **124**, 1853–1867 (2014).
39. Okada, N. *et al.* A positive feedback between p53 and miR-34 miRNAs mediates tumor suppression. *Genes Dev.* **28**, 438–450 (2014).
40. Li, L. *et al.* MiR-34a inhibits proliferation and migration of breast cancer through down-regulation of Bcl-2 and SIRT1. *Clin. Exp. Med.* **13**, 109–117 (2013).
41. Tarasov, V. *et al.* Differential regulation of microRNAs by p53 revealed by massively parallel sequencing: miR-34a is a p53 target that induces apoptosis and G1-arrest. *Cell Cycle* **6**, 1586–1593 (2007).
42. Chang, T. C. *et al.* Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. *Mol. Cell* **26**, 745–752 (2007).
43. Raver-Shapira, N. *et al.* Transcriptional activation of miR-34a contributes to p53-mediated apoptosis. *Mol. Cell* **26**, 731–743 (2007).
44. Misso, G. *et al.* Mir-34: a new weapon against cancer? *Mol. Ther. Nucleic Acids* **3**, e194 (2014).
45. Pardoll, D. M. The blockade of immune checkpoints in cancer immunotherapy. *Nat. Rev. Cancer* **12**, 252–264 (2012).
46. Wang, X. *et al.* Tumor suppressor miR-34a targets PD-L1 and functions as a potential immunotherapeutic target in acute myeloid leukemia. *Cell. Signal.* **27**, 443–452 (2015).
47. Cortez, M. A. *et al.* PDL1 regulation by p53 via miR-34. *J. Natl Cancer Inst.* **108,** djv303 (2016).
48. Yu, F. *et al. let-7* regulates self renewal and tumorigenicity of breast cancer cells. *Cell* **131**, 1109–1123 (2007).
49. Johnson, C. D. *et al.* The *let-7* microRNA represses cell proliferation pathways in human cells. *Cancer Res.* **67**, 7713–7722 (2007).
50. Brueckner, B. *et al.* The human *let-7a-3* locus contains an epigenetically regulated microRNA gene with oncogenic function. *Cancer Res.* **67**, 1419–1423 (2007).
51. Iliopoulos, D., Hirsch, H. A. & Struhl, K. An epigenetic switch involving NF-κB, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. *Cell* **139**, 693–706 (2009).
52. Sung, S. Y. *et al.* Loss of let-7 microRNA upregulates IL-6 in bone marrow-derived mesenchymal stem cells triggering a reactive stromal response to prostate cancer. *PLoS ONE* **8**, e71637 (2013).
53. Johnson, S. M. *et al. RAS* is regulated by the *let-7* microRNA family. *Cell* **120**, 635–647 (2005).
54. Newman, M. A., Thomson, J. M. & Hammond, S. M. Lin-28 interaction with the Let-7 precursor loop mediates regulated microRNA processing. *RNA* **14**, 1539–1549 (2008).
55. Gregory, P. A. *et al.* The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat. Cell Biol.* **10**, 593–601 (2008).
56. De Craene, B. & Berx, G. Regulatory networks defining EMT during cancer initiation and progression. *Nat. Rev. Cancer* **13**, 97–110 (2013).
57. Korpal, M., Lee, E. S., Hu, G. & Kang, Y. The miR-200 family inhibits epithelial–mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2. *J. Biol. Chem.* **283**, 14910–14914 (2008).
58. Park, S. M., Gaur, A. B., Lengyel, E. & Peter, M. E. The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. *Genes Dev.* **22**, 894–907 (2008).
59. Burk, U. *et al.* A reciprocal repression between ZEB1 and members of the miR-200 family promotes EMT and invasion in cancer cells. *EMBO Rep.* **9**, 582–589 (2008).
60. Gregory, P. A. *et al.* An autocrine TGF-β/ZEB/miR-200 signaling network regulates establishment and maintenance of epithelial–mesenchymal transition. *Mol. Biol. Cell* **22**, 1686–1698 (2011).
61. Pecot, C. V. *et al.* Tumour angiogenesis regulation by the miR-200 family. *Nat. Commun.* **4**, 2427 (2013).
62. Pekarsky, Y. & Croce, C. M. Role of *miR-15/16* in CLL. *Cell Death Differ.* **22**, 6–11 (2015).
63. Klein, U. *et al.* The *DLEU2/miR-15a/16-1* cluster controls B cell proliferation and its deletion leads to chronic lymphocytic leukemia. *Cancer Cell* **17**, 28–40 (2010).
64. Yang, D. *et al.* Integrated analyses identify a master microRNA regulatory network for the mesenchymal subtype in serous ovarian cancer. *Cancer Cell* **23**, 186–199 (2013).
65. Liu, G. *et al.* Augmentation of response to chemotherapy by microRNA-506 through regulation of RAD51 in serous ovarian cancers. *J. Natl Cancer Inst.* **107,** djv108 (2015).
66. Liu, G. *et al.* MiR-506 suppresses proliferation and induces senescence by directly targeting the CDK4/6–FOXM1 axis in ovarian cancer. *J. Pathol.* **233**, 308–318 (2014).
67. Keklikoglou, I. *et al.* MicroRNA-520/373 family functions as a tumor suppressor in estrogen receptor negative breast cancer by targeting NF-κB and TGF-β signaling pathways. *Oncogene* **31**, 4150–4163 (2012).
68. Nishimura, M. *et al.* Therapeutic synergy between microRNA and siRNA in ovarian cancer treatment. *Cancer Discov.* **3**, 1302–1315 (2013).
69. Landen, C. N. Jr *et al.* Therapeutic *EphA2* gene targeting *in vivo* using neutral liposomal small interfering RNA delivery. *Cancer Res.* **65**, 6910–6918 (2005).
First report of using neutral liposome DOPC for the delivery of small interfering RNAs (siRNAs) to tumours.
70. Asangani, I. A. *et al.* MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. *Oncogene* **27**, 2128–2136 (2008).
71. Frankel, L. B. *et al.* Programmed cell death 4 (PDCD4) is an important functional target of the microRNA miR-21 in breast cancer cells. *J. Biol. Chem.* **283**, 1026–1033 (2008).
72. Yan, L. X. *et al.* MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. *RNA* **14**, 2348–2360 (2008).
73. Li, Q. *et al.* MiR-21/Smad 7 signaling determines TGF-β1-induced CAF formation. *Sci. Rep.* **3**, 2038 (2013).
74. Medina, P. P., Nolde, M. & Slack, F. J. OncomiR addiction in an *in vivo* model of microRNA-21-induced pre-B-cell lymphoma. *Nature* **467**, 86–90 (2010).
Reports the concept of oncomiR addiction, whereby cancers tend to have a dependence on oncogenic miRNA expression.
75. Hatley, M. E. *et al.* Modulation of K-Ras-dependent lung tumorigenesis by microRNA-21. *Cancer Cell* **18**, 282–293 (2010).
76. Krichevsky, A. M. & Gabriely, G. miR-21: a small multi-faceted RNA. *J. Cell. Mol. Med.* **13**, 39–53 (2009).
77. Campbell, J. D. *et al.* Distinct patterns of somatic genome alterations in lung adenocarcinomas and squamous cell carcinomas. *Nat. Genet.* **48**, 607–616 (2016).
78. Fujita, S. *et al. miR-21* gene expression triggered by AP-1 is sustained through a double-negative feedback mechanism. *J. Mol. Biol.* **378**, 492–504 (2008).
79. Iliopoulos, D., Jaeger, S. A., Hirsch, H. A., Bulyk, M. L. & Struhl, K. STAT3 activation of miR-21 and miR-181b-1 via PTEN and CYLD are part of the epigenetic switch linking inflammation to cancer. *Mol. Cell* **39**, 493–506 (2010).
80. Gironella, M. *et al.* Tumor protein 53-induced nuclear protein 1 expression is repressed by miR-155, and its restoration inhibits pancreatic tumor development. *Proc. Natl Acad. Sci. USA* **104**, 16170–16175 (2007).
81. O'Connell, R. M., Chaudhuri, A. A., Rao, D. S. & Baltimore, D. Inositol phosphatase SHIP1 is a primary target of miR-155. *Proc. Natl Acad. Sci. USA* **106**, 7113–7118 (2009).
82. Faraoni, I., Antonetti, F. R., Cardone, J. & Bonmassar, E. *miR-155* gene: a typical multifunctional microRNA. *Biochim. Biophys. Acta* **1792**, 497–505 (2009).
83. Tili, E., Croce, C. M. & Michaille, J. J. miR-155: on the crosstalk between inflammation and cancer. *Int. Rev. Immunol.* **28**, 264–284 (2009).

84. Tili, E. *et al.* Mutator activity induced by microRNA-155 (miR-155) links inflammation and cancer. *Proc. Natl Acad. Sci. USA* **108**, 4908–4913 (2011).
85. Babar, I. A. *et al.* Nanoparticle-based therapy in an *in vivo* microRNA-155 (miR-155)-dependent mouse model of lymphoma. *Proc. Natl Acad. Sci. USA* **109**, E1695–E1704 (2012).
86. Kong, W. *et al.* Upregulation of miRNA-155 promotes tumour angiogenesis by targeting VHL and is associated with poor prognosis and triple-negative breast cancer. *Oncogene* **33**, 679–689 (2014).
87. Kulshreshtha, R. *et al.* A microRNA signature of hypoxia. *Mol. Cell. Biol.* **27**, 1859–1867 (2007). **Reports that miR-210, a hypoxia marker miRNA, is significantly upregulated during hypoxia exposure.**
88. Puissegur, M. P. *et al.* miR-210 is overexpressed in late stages of lung cancer and mediates mitochondrial alterations associated with modulation of HIF-1 activity. *Cell Death Differ.* **18**, 465–478 (2011).
89. Yang, W. *et al.* Downregulation of miR-210 expression inhibits proliferation, induces apoptosis and enhances radiosensitivity in hypoxic human hepatoma cells *in vitro*. *Exp. Cell Res.* **318**, 944–954 (2012).
90. Fasanaro, P. *et al.* MicroRNA-210 modulates endothelial cell response to hypoxia and inhibits the receptor tyrosine kinase ligand Ephrin-A3. *J. Biol. Chem.* **283**, 15878–15883 (2008).
91. Crosby, M. E., Kulshreshtha, R., Ivan, M. & Glazer, P. M. MicroRNA regulation of DNA repair gene expression in hypoxic stress. *Cancer Res.* **69**, 1221–1229 (2009).
92. Mogilyansky, E. & Rigoutsos, I. The miR-17/92 cluster: a comprehensive update on its genomics, genetics, functions and increasingly important and numerous roles in health and disease. *Cell Death Differ.* **20**, 1603–1614 (2013).
93. O'Donnell, K. A., Wentzel, E. A., Zeller, K. I., Dang, C. V. & Mendell, J. T. c-Myc-regulated microRNAs modulate E2F1 expression. *Nature* **435**, 839–843 (2005).
94. Koralov, S. B. *et al.* Dicer ablation affects antibody diversity and cell survival in the B lymphocyte lineage. *Cell* **132**, 860–874 (2008).
95. Dews, M. *et al.* Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. *Nat. Genet.* **38**, 1060–1065 (2006).
96. de Pontual, L. *et al.* Germline deletion of the miR-17 approximately 92 cluster causes skeletal and growth defects in humans. *Nat. Genet.* **43**, 1026–1030 (2011).
97. Marcelis, C. L. *et al.* Genotype–phenotype correlations in MYCN-related Feingold syndrome. *Hum. Mutat.* **29**, 1125–1132 (2008).
98. Ma, L., Teruya-Feldstein, J. & Weinberg, R. A. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature* **449**, 682–688 (2007).
99. Bloomston, M. *et al.* MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. *JAMA* **297**, 1901–1908 (2007).
100. Saldanha, G. *et al.* microRNA-10b is a prognostic biomarker for melanoma. *Mod. Pathol.* **29**, 112–121 (2016).
101. Ma, L. *et al.* Therapeutic silencing of miR-10b inhibits metastasis in a mouse mammary tumor model. *Nat. Biotechnol.* **28**, 341–347 (2010). **A study that uses antimiR against miR-10b to suppress miR-10b function and demonstrates significant reduction in breast cancer metastasis.**
102. Nakayama, I. *et al.* Loss of HOXD10 expression induced by upregulation of miR-10b accelerates the migration and invasion activities of ovarian cancer cells. *Int. J. Oncol.* **43**, 63–71 (2013).
103. Garofalo, M. *et al. miR-221&222* regulate TRAIL resistance and enhance tumorigenicity through PTEN and TIMP3 downregulation. *Cancer Cell* **16**, 498–509 (2009).
104. le Sage, C. *et al.* Regulation of the $p27^{Kip1}$ tumor suppressor by miR-221 and miR-222 promotes cancer cell proliferation. *EMBO J.* **26**, 3699–3708 (2007).
105. Pineau, P. *et al.* miR-221 overexpression contributes to liver tumorigenesis. *Proc. Natl Acad. Sci. USA* **107**, 264–269 (2010).
106. Wiggins, J. F. *et al.* Development of a lung cancer therapeutic based on the tumor suppressor microRNA-34. *Cancer Res.* **70**, 5923–5930 (2010).
107. Trang, P. *et al.* Systemic delivery of tumor suppressor microRNA mimics using a neutral lipid emulsion inhibits lung tumors in mice. *Mol. Ther.* **19**, 1116–1122 (2011).
108. Pramanik, D. *et al.* Restitution of tumor suppressor microRNAs using a systemic nanovector inhibits pancreatic cancer growth in mice. *Mol. Cancer Ther.* **10**, 1470–1480 (2011).
109. Kasinski, A. L. & Slack, F. J. miRNA-34 prevents cancer initiation and progression in a therapeutically resistant K-ras and p53-induced mouse model of lung adenocarcinoma. *Cancer Res.* **72**, 5576–5587 (2012).
110. Stahlhut, C. & Slack, F. J. Combinatorial action of microRNAs *let-7* and miR-34 effectively synergizes with erlotinib to suppress non-small cell lung cancer cell proliferation. *Cell Cycle* **14**, 2171–2180 (2015).
111. Cortez, M. A. *et al.* Therapeutic delivery of miR-200c enhances radiosensitivity in lung cancer. *Mol. Ther.* **22**, 1494–1503 (2014).
112. Kota, J. *et al.* Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. *Cell* **137**, 1005–1017 (2009).
113. Ji, J. *et al.* MicroRNA expression, survival, and response to interferon in liver cancer. *N. Engl. J. Med.* **361**, 1437–1447 (2009).
114. Calin, G. A. *et al.* MiR-15a and miR-16-1 cluster functions in human leukemia. *Proc. Natl Acad. Sci. USA* **105**, 5166–5171 (2008).
115. Reid, G. *et al.* Abstract 3976: targeted delivery of a synthetic microRNA-based mimic as an approach to cancer therapy. *Cancer Res.* **75**, abstr. 3976 (2015).
116. Gabriely, G. *et al.* Human glioma growth is controlled by microRNA-10b. *Cancer Res.* **71**, 3563–3572 (2011).
117. Yoo, B. *et al.* Combining miR-10b-targeted nanotherapy with low-dose doxorubicin elicits durable regressions of metastatic breast cancer. *Cancer Res.* **75**, 4407–4415 (2015).
118. Park, J. K. *et al.* miR-221 silencing blocks hepatocellular carcinoma and promotes survival. *Cancer Res.* **71**, 7608–7616 (2011).
119. Cheng, C. J. *et al.* MicroRNA silencing for cancer therapy targeted to the tumour microenvironment. *Nature* **518**, 107–110 (2015).
120. Reshetnyak, Y. K., Andreev, O. A., Lehnert, U. & Engelman, D. M. Translocation of molecules into cells by pH-dependent insertion of a transmembrane helix. *Proc. Natl Acad. Sci. USA* **103**, 6460–6465 (2006).
121. Jopling, C. L., Yi, M., Lancaster, A. M., Lemon, S. M. & Sarnow, P. Modulation of hepatitis C virus RNA abundance by a liver-specific microRNA. *Science* **309**, 1577–1581 (2005).
122. Thibault, P. A. *et al.* Regulation of hepatitis C virus genome replication by Xrn1 and microRNA-122 binding to individual sites in the 5′ untranslated region. *J. Virol.* **89**, 6294–6311 (2015).
123. Luna, J. M. *et al.* Hepatitis C virus RNA functionally sequesters miR-122. *Cell* **160**, 1099–1110 (2015). **A study that records the functional role of HCV RNA sequestration of miR-122 and relevance to therapy (read along with references 124 and 125).**
124. Elmen, J. *et al.* Antagonism of microRNA-122 in mice by systemically administered LNA-antimiR leads to up-regulation of a large set of predicted target mRNAs in the liver. *Nucleic Acids Res.* **36**, 1153–1162 (2008).
125. Elmen, J. *et al.* LNA-mediated microRNA silencing in non-human primates. *Nature* **452**, 896–899 (2008).
126. Hsu, S. H. *et al.* Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver. *J. Clin. Invest.* **122**, 2871–2883 (2012).
127. Tsai, W. C. *et al.* MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. *J. Clin. Invest.* **122**, 2884–2897 (2012).
128. Krutzfeldt, J. *et al.* Silencing of microRNAs *in vivo* with 'antagomirs'. *Nature* **438**, 685–689 (2005).
129. Cheng, Y. & Zhang, C. MicroRNA-21 in cardiovascular disease. *J. Cardiovasc. Transl. Res.* **3**, 251–255 (2010).
130. Thum, T. *et al.* MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. *Nature* **456**, 980–984 (2008).
131. Chau, B. N. *et al.* MicroRNA-21 promotes fibrosis of the kidney by silencing metabolic pathways. *Sci. Transl. Med.* **4**, 121ra18 (2012).
132. Xin, M. *et al.* MicroRNAs miR-143 and miR-145 modulate cytoskeletal dynamics and responsiveness of smooth muscle cells to injury. *Genes Dev.* **23**, 2166–2178 (2009).
133. Boettger, T. *et al.* Acquisition of the contractile phenotype by murine arterial smooth muscle cells depends on the *Mir143/145* gene cluster. *J. Clin. Invest.* **119**, 2634–2647 (2009).
134. Davis-Dusenbery, B. N. *et al.* Down-regulation of Kruppel-like factor-4 (KLF4) by microRNA-143/145 is critical for modulation of vascular smooth muscle cell phenotype by transforming growth factor-β and bone morphogenetic protein 4. *J. Biol. Chem.* **286**, 28097–28110 (2011).
135. Cordes, K. R. *et al.* miR-145 and miR-143 regulate smooth muscle cell fate and plasticity. *Nature* **460**, 705–710 (2009).
136. Ikeda, S. *et al.* MicroRNA-1 negatively regulates expression of the hypertrophy-associated calmodulin and *Mef2a* genes. *Mol. Cell. Biol.* **29**, 2193–2204 (2009).
137. Shan, Z. X. *et al.* Upregulated expression of miR-1/miR-206 in a rat model of myocardial infarction. *Biochem. Biophys. Res. Commun.* **381**, 597–601 (2009).
138. Grueter, C. E. *et al.* A cardiac microRNA governs systemic energy homeostasis by regulation of MED13. *Cell* **149**, 671–683 (2012).
139. Montgomery, R. L. *et al.* Therapeutic inhibition of miR-208a improves cardiac function and survival during heart failure. *Circulation* **124**, 1537–1547 (2011).
140. Montgomery, R. L. *et al.* MicroRNA mimicry blocks pulmonary fibrosis. *EMBO Mol. Med.* **6**, 1347–1356 (2014).
141. Rayner, K. J. *et al.* MiR-33 contributes to the regulation of cholesterol homeostasis. *Science* **328**, 1570–1573 (2010).
142. Davalos, A. *et al.* miR-33a/b contribute to the regulation of fatty acid metabolism and insulin signaling. *Proc. Natl Acad. Sci. USA* **108**, 9232–9237 (2011).
143. Rayner, K. J. *et al.* Inhibition of miR-33a/b in non-human primates raises plasma HDL and lowers VLDL triglycerides. *Nature* **478**, 404–407 (2011).
144. Goedeke, L. *et al.* Long-term therapeutic silencing of miR-33 increases circulating triglyceride levels and hepatic lipid accumulation in mice. *EMBO Mol. Med.* **6**, 1133–1141 (2014).
145. Belgardt, B. F. *et al.* The microRNA-200 family regulates pancreatic beta cell survival in type 2 diabetes. *Nat. Med.* **21**, 619–627 (2015).
146. McArthur, K., Feng, B., Wu, Y., Chen, S. & Chakrabarti, S. MicroRNA-200b regulates vascular endothelial growth factor-mediated alterations in diabetic retinopathy. *Diabetes* **60**, 1314–1323 (2011).
147. Wang, B. *et al.* E-Cadherin expression is regulated by miR-192/215 by a mechanism that is independent of the profibrotic effects of transforming growth factor-β. *Diabetes* **59**, 1794–1802 (2010).
148. Slusarz, A. & Pulakat, L. The two faces of miR-29. *J. Cardiovasc. Med. (Hagerstown)* **16**, 480–490 (2015).
149. Roggli, E. *et al.* Changes in microRNA expression contribute to pancreatic β-cell dysfunction in prediabetic NOD mice. *Diabetes* **61**, 1742–1751 (2012).
150. Maurer, B. *et al.* MicroRNA-29, a key regulator of collagen expression in systemic sclerosis. *Arthritis Rheum.* **62**, 1733–1743 (2010).
151. Janssen, H. L. *et al.* Treatment of HCV infection by targeting microRNA. *N. Engl. J. Med.* **368**, 1685–1694 (2013).
152. Ottosen, S. *et al. In vitro* antiviral activity and preclinical and clinical resistance profile of miravirsen, a novel anti-hepatitis C virus therapeutic targeting the human factor miR-122. *Antimicrob. Agents Chemother.* **59**, 599–608 (2015).
153. Hong. D. S. *et al.* MRX34, a liposomal miR-34 mimic, in patients with advanced solid tumors: Final dose-escalation results from a first-in-human phase I trial of microRNA therapy. *J. Clin. Oncol.* **34**, (Suppl), abstr. 2508 (2015).
154. Beg, M. S. *et al.* Abstract C43: safety, tolerability, and clinical activity of MRX34, the first-in-class liposomal miR-34 mimic, in patients with advanced solid tumors. *Mol. Cancer Ther.* **14**, abstr. C43 (2015). **Highlights the clinical safety and activity data of MRX34, a miR-34 mimic-based therapy against cancers.**
155. van Zandwijk, N. *et al.* P1.02: MesomiR 1: a phase I study of TargomiRs in patients with refractory malignant pleural mesothelioma (MPM) and lung cancer (NSCLC). *Ann. Oncol.* **26** (Suppl. 2), ii16 (2015).
156. Trajkovski, M. *et al.* MicroRNAs 103 and 107 regulate insulin sensitivity. *Nature* **474**, 649–653 (2011).

157. Cheng, C. J. & Slack, F. J. The duality of oncomiR addiction in the maintenance and treatment of cancer. *Cancer J.* **18**, 232–237 (2012).
158. Rupaimoole, R., Han, H. D., Lopez-Berestein, G. & Sood, A. K. MicroRNA therapeutics: principles, expectations, and challenges. *Chin. J. Cancer* **30**, 368–370 (2011).
159. Vaupel, P. & Mayer, A. Hypoxia in cancer: significance and impact on clinical outcome. *Cancer Metastasis Rev.* **26**, 225–239 (2007).
160. Rupaimoole, R. *et al.* Hypoxia-mediated downregulation of miRNA biogenesis promotes tumour progression. *Nat. Commun.* **5,** 5202 (2014).
161. Imig, J. *et al.* miR-CLIP capture of a miRNA targetome uncovers a lincRNA H19–miR-106a interaction. *Nat. Chem. Biol.* **11**, 107–114 (2015).
162. Chou, C. H. *et al.* miRTarBase 2016: updates to the experimentally validated miRNA-target interactions database. *Nucleic Acids Res.* **44**, D239–D247 (2016).
163. Li, J. H., Liu, S., Zhou, H., Qu, L. H. & Yang, J. H. starBase v2.0: decoding miRNA–ceRNA, miRNA–ncRNA and protein–RNA interaction networks from large-scale CLIP-Seq data. *Nucleic Acids Res.* **42**, D92–D97 (2014).
164. Agarwal, V., Bell, G. W., Nam, J. W. & Bartel, D. P. Predicting effective microRNA target sites in mammalian mRNAs. *eLife* **4,** e05005 (2015).
165. Eulalio, A. *et al.* Functional screening identifies miRNAs inducing cardiac regeneration. *Nature* **492**, 376–381 (2012).
166. Kovalchuk, O. *et al.* Involvement of microRNA-451 in resistance of the MCF-7 breast cancer cells to chemotherapeutic drug doxorubicin. *Mol. Cancer Ther.* **7**, 2152–2159 (2008).
167. Sun, L. *et al.* MiR-200b and miR-15b regulate chemotherapy-induced epithelial–mesenchymal transition in human tongue cancer cells by targeting BMI1. *Oncogene* **31**, 432–445 (2012).
168. Geary, R. S. *et al.* Pharmacokinetic properties of 2'-O-(2-methoxyethyl)-modified oligonucleotide analogs in rats. *J. Pharmacol. Exp. Ther.* **296**, 890–897 (2001).
169. Esau, C. *et al.* miR-122 regulation of lipid metabolism revealed by *in vivo* antisense targeting. *Cell. Metab.* **3**, 87–98 (2006).
170. Reshetnyak, Y. K., Andreev, O. A., Segala, M., Markin, V. S. & Engelman, D. M. Energetics of peptide (pHLIP) binding to and folding across a lipid bilayer membrane. *Proc. Natl Acad. Sci. USA* **105**, 15340–15345 (2008).
171. Kulkarni, R. K., Moore, E. G., Hegyeli, A. F. & Leonard, F. Biodegradable poly(lactic acid) polymers. *J. Biomed. Mater. Res.* **5**, 169–181 (1971).
172. Blum, J. S. & Saltzman, W. M. High loading efficiency and tunable release of plasmid DNA encapsulated in submicron particles fabricated from PLGA conjugated with poly-L-lysine. *J. Control. Release* **129**, 66–72 (2008).
173. Yang, X. Z. *et al.* Systemic delivery of siRNA with cationic lipid assisted PEG-PLA nanoparticles for cancer therapy. *J. Control. Release* **156**, 203–211 (2011).
174. Ozpolat, B., Sood, A. K. & Lopez-Berestein, G. Nanomedicine based approaches for the delivery of siRNA in cancer. *J. Intern. Med.* **267**, 44–53 (2010).
175. Joshi, H. P. *et al.* Dynamin 2 along with microRNA-199a reciprocally regulate hypoxia-inducible factors and ovarian cancer metastasis. *Proc. Natl Acad. Sci. USA* **111**, 5331–5336 (2014).
176. MacDiarmid, J. A. *et al.* Bacterially derived 400 nm particles for encapsulation and cancer cell targeting of chemotherapeutics. *Cancer Cell* **11**, 431–445 (2007).
177. Taylor, K. *et al.* Nanocell targeting using engineered bispecific antibodies. *MAbs* **7**, 53–65 (2015).
178. Akhtar, S. & Benter, I. F. Nonviral delivery of synthetic siRNAs *in vivo*. *J. Clin. Invest.* **117**, 3623–3632 (2007).
179. Duncan, R. & Izzo, L. Dendrimer biocompatibility and toxicity. *Adv. Drug Deliv. Rev.* **57**, 2215–2237 (2005).
180. Gonzalez, H., Hwang, S. J. & Davis, M. E. New class of polymers for the delivery of macromolecular therapeutics. *Bioconjug. Chem.* **10**, 1068–1074 (1999).
181. Davis, M. E. *et al.* Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. *Nature* **464**, 1067–1070 (2010). **Study reports data on first in-human clinical trial involving siRNA-based therapeutics against cancer.**
182. Kim, S. H., Jeong, J. H., Lee, S. H., Kim, S. W. & Park, T. G. PEG conjugated VEGF siRNA for anti-angiogenic gene therapy. *J. Control. Release* **116**, 123–129 (2006).
183. Ragelle, H., Vandermeulen, G. & Preat, V. Chitosan-based siRNA delivery systems. *J. Control. Release* **172**, 207–218 (2013).
184. Nair, J. K. *et al.* Multivalent *N*-acetylgalactosamine-conjugated siRNA localizes in hepatocytes and elicits robust RNAi-mediated gene silencing. *J. Am. Chem. Soc.* **136**, 16958–16961 (2014).
185. Dimitrova, N. *et al.* Stromal expression of miR-143/145 promotes neoangiogenesis in lung cancer development. *Cancer Discov.* **6**, 188–201 (2016).
186. Suzuki, H. I., Katsura, A., Matsuyama, H. & Miyazono, K. MicroRNA regulons in tumor microenvironment. *Oncogene* **34**, 3085–3094 (2015).
187. Frediani, J. N. & Fabbri, M. Essential role of miRNAs in orchestrating the biology of the tumor microenvironment. *Mol. Cancer* **15**, 42 (2016).
188. Bronisz, A. *et al.* Reprogramming of the tumour microenvironment by stromal PTEN-regulated miR-320. *Nat. Cell Biol.* **14**, 159–167 (2011).
189. Mitra, A. K. *et al.* MicroRNAs reprogram normal fibroblasts into cancer-associated fibroblasts in ovarian cancer. *Cancer Discov.* **2**, 1100–1108 (2012).
190. Ibrahim, A. F. *et al.* MicroRNA replacement therapy for miR-145 and miR-33a is efficacious in a model of colon carcinoma. *Cancer Res.* **71**, 5214–5224 (2011).
191. Pramanik, D. *et al.* Restitution of tumor suppressor microRNAs using a systemic nanovector inhibits pancreatic cancer growth in mice. *Mol. Cancer Ther.* **10**, 1470–1480 (2011).
192. Putta, S. *et al.* Inhibiting microRNA-192 ameliorates renal fibrosis in diabetic nephropathy. *J. Am. Soc. Nephrol.* **23**, 458–469 (2012).
193. Long, J., Wang, Y., Wang, W., Chang, B. H. & Danesh, F. R. MicroRNA-29c is a signature microRNA under high glucose conditions that targets Sprouty homolog 1, and its *in vivo* knockdown prevents progression of diabetic nephropathy. *J. Biol. Chem.* **286**, 11837–11848 (2011).
194. Hullinger, T. G. *et al.* Inhibition of miR-15 protects against cardiac ischemic injury. *Circ. Res.* **110**, 71–81 (2012).

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Competing interests statement
The authors declare competing interests: see Web version for details.

DATABASES
ClinicalTrials.gov: http://www.clinicaltrials.gov
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FURTHER INFORMATION
Mirna Therapeutics Halts Phase 1 Clinical Study of MRX34: http://investor.mirnarx.com/releasedetail.cfm?ReleaseID=990204
Regulus Therapeutics, press release dated 9 February 2015: http://ir.regulusrx.com/releasedetail.cfm?ReleaseID=895314
Regulus Therapeutics, press release dated 7 June 2016: http://ir.regulusrx.com/releasedetail.cfm?ReleaseID=974583
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