MicroRNAs and other non-coding RNAs as targets for anticancer drug development

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Abstract | The first cancer-targeted microRNA (miRNA) drug — MRX34, a liposome-based miR-34 mimic — entered Phase I clinical trials in patients with advanced hepatocellular carcinoma in April 2013, and miRNA therapeutics are attracting special attention from both academia and biotechnology companies. Although miRNAs are the most studied non-coding RNAs (ncRNAs) to date, the importance of long non-coding RNAs (lncRNAs) is increasingly being recognized. Here, we summarize the roles of miRNAs and lncRNAs in cancer, with a focus on the recently identified novel mechanisms of action, and discuss the current strategies in designing ncRNA-targeting therapeutics, as well as the associated challenges.

Non-coding RNAs (ncRNAs). RNA molecules that are not translated into protein.

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Non-coding RNAs (ncRNAs) comprise multiple classes of RNA transcripts that are not transcribed into proteins but have been shown to regulate the transcription, stability or translation of protein-coding genes in the mammalian genome[1,2]. To date, the most studied ncRNAs are microRNAs (miRNAs; usually 19–24 nucleotides in length), but many other classes of experimentally identified ncRNAs with various lengths and characteristics have been reported in the literature (BOX 1).

The involvement of miRNAs in the development of cancer was first demonstrated in 2002 (REF. 3); since then, the role of miRNAs has been intensively investigated in multiple human diseases[4]. During the past decade, over 25,000 papers deposited on PubMed have reported on various aspects of miRNA genomics, biogenesis, mechanisms of action, pathway involvement, phenotypes in experimental models and disease abnormalities. About 40% of these publications have focused on the role of miRNAs in cancer. These studies unveiled a novel mechanism of post-transcriptional regulation that is profoundly altered in malignant cells[5]. Using high-throughput techniques such as expression microarrays or next-generation sequencing, it has been shown that miRNAs are dysregulated in almost all types of human cancer[6,7], and specific signatures of aberrantly expressed miRNAs harbour diagnostic, prognostic and theranostic implications[8–11]. In addition, miRNA expression patterns allow an accurate discrimination between different types of cancer and the identification of the tissue of origin of poorly differentiated tumours[6].

More recently, other types of ncRNAs — such as long non-coding RNAs (lncRNAs) — were found to have dynamic roles in transcriptional and translational regulation[1], and to be involved in several human diseases including cancer[8,12]. These findings suggest that multiple types of ncRNAs functionally participate in normal physiological activities and disease phenotypes (TABLE 1). The fact that about 75% of the human genome is transcribed into RNA, whereas only 3% is transcribed into protein-coding mRNAs[13–15], indicates that the number of ncRNAs is potentially much higher than that of protein-coding genes. Therefore, ncRNAs could represent goldmines for basic research, biomarker discovery and therapeutic applications[10,16–21].

Here, we summarize recent insights into the physiological function of miRNAs and their involvement in disease, focusing on cancer, and discuss how these insights can be used for the development of new anticancer drugs. We also discuss emerging insights into the role of lncRNAs and their potential as targets for novel treatment paradigms.

miRNA generation and function

Mature miRNAs are evolutionally conserved single-stranded RNAs. The generation of mature miRNAs is a multi-step process that starts with the initial transcription of their genes by RNA polymerase II. This results in long, capped and polyadenylated primary miRNAs, which can be several hundreds to several thousands of nucleotides long. These primary transcripts are processed by the ribonuclease (RNase) III Drosha–DGCR8

Box 1 | **Various types of non-coding RNAs**

- MicroRNAs (miRNAs) are 19–24-nucleotide-long, single-stranded RNAs that are initially transcribed from the genome as primary miRNAs and processed into precursor and mature forms through a biogenesis machinery that includes the enzymes Drosha and Dicer. miRNAs exert biological functions by regulating the transcription and/or translation of protein-coding genes.
- Endogenous small interfering RNAs (endo-siRNAs) are ~20-nucleotide-long single-stranded RNAs that are processed from several sources of endogenous double-stranded RNA, including transposable elements, *cis*-natural antisense transcripts (NATs), *trans*-NATs and hairpin RNA transcripts. Endo-siRNAs load onto Argonaute 2 and repress transposon transcripts or endogenous mRNAs[133].
- PIWI-interacting RNAs (piRNAs) are single-stranded RNAs that are 21–35 nucleotides in length and have roles in germline transposon silencing and gametogenesis[134].
- Small nucleolar RNAs (snoRNAs) are 60–300-nucleotide-long RNAs that have roles in the modification of ribosomal RNAs[135].
- Sno-derived RNAs (sdRNAs) are miRNA-like RNAs that are 20–24 nucleotides in length and originate from H/ACA box snoRNAs, or RNAs that are ~17 nucleotides or >27 nucleotides in length and are derived from C/D box snoRNAs[136].
- Transcription initiation RNAs (tiRNAs) are 18-nucleotide-long RNAs that are localized to the nucleus and originate from sequences immediately downstream of the RNA polymerase II transcription start site[137].
- miRNA-offset RNAs (moRNAs) are produced from human miRNA precursors, but their levels of expression are considerably lower and not correlated with the expression of associated miRNAs[138].
- Long non-coding RNAs (lncRNAs) are non-protein-coding RNA transcripts that are longer than 200 nucleotides and have multiple functions and mechanisms of action[1,12]. Several types of lncRNAs are listed below.
- NATs are endogenous RNA molecules with partial or full sequence complementarity to other transcripts. There are two types of NATs: *cis*-NATs are transcribed from the same genomic loci as their sense transcripts but from the opposite DNA strand, whereas *trans*-NATs originate from genomic regions that are distinct from those encoding their targeted sense transcripts[105,139].
- Circular RNAs (circRNAs) are endogenous RNAs with covalently linked ends that can serve as miRNA sponges. One example is that of a ~1,500-nucleotide-long circRNA, which is predominately located in the human and mouse brain, that contains multiple binding sites for miR-7 and sequesters this miRNA[103,140].
- Long intergenic non-coding RNAs (lincRNAs) are transcribed from non-coding DNA sequences located between protein-coding genes[141].
- Long enhancer non-coding RNAs are lncRNAs with enhancer-like functions; they regulate neighbouring protein-coding genes[107,142].
- Transcribed ultraconserved regions (T-UCRs) are lncRNAs with significant biological function that interact with microRNAs and overlap with the genomic ultraconserved regions[106,115].
- Pseudogenes are genomic loci that resemble real genes, but were previously considered biologically irrelevant because they contain mutations that abrogate their translation into functional proteins. However, it has become apparent that pseudogenes are transcribed into lncRNAs, and have functional roles in regulating RNA expression.

(DiGeorge syndrome critical region 8) nuclear complex into hairpin structure precursor miRNAs of 60–100 nucleotides, which are subsequently transported from the nucleus to the cytoplasm by exportin 5 and further cleaved by the RNase enzyme Dicer into double-stranded miRNAs. The two strands are separated by helicases, and the mature strand is incorporated into the RNA-induced silencing complex (RISC). Typically, mature miRNAs regulate gene expression through sequence-specific binding to the 3′ untranslated region (3′ UTR) of a target mRNA, but several lines of evidence indicate that miRNAs can also bind to other regions of a target mRNA[22,23]. The miRNA–mRNA interaction usually causes translational repression and/or mRNA cleavage and thus reduces the final protein output.

Overall, this 'traditional' understanding of miRNAs as negative regulators of gene expression has recently been challenged by the discovery of new and unexpected mechanisms of action of miRNAs (BOX 2). This includes evidence that miRNAs can also increase the translation of a target mRNA by recruiting protein complexes to the AU-rich elements of the mRNA[24], or they can indirectly increase the target protein output by de-repressing mRNA translation by interacting with proteins that block the translation of the target gene[25]. There is also evidence to indicate that miRNAs can cause global protein synthesis by enhancing ribosome biogenesis[26], or switch the regulation from repression to activation of target gene translation in conditions of cell cycle arrest[24].

In addition to functioning within cells, miRNAs are abundant in the bloodstream and can act at neighbouring cells and at more distant sites within the body in a hormone-like fashion, which indicates that they can mediate both short- and long-range cell–cell communication[27,28]. miRNAs, together with RNA-binding proteins (such as nucleophosmin 1 and Argonaute 2), can be packaged and transported extracellularly by exosomes or microvesicles[29–32]. Likewise, precursor miRNAs inside the donor cell can be stably exported in conjunction with RNA-binding proteins or by binding to high-density lipoprotein[31]. Passive leakage from cells, owing to injury, chronic inflammation, apoptosis or necrosis, or from cells with short half-lives (such as platelets), is thought to be another way of release. Circulating miRNAs enter the bloodstream and are taken up by the recipient cells via endocytosis and further bind to intracellular proteins such as Toll-like receptors (TLRs)[27]. It is hypothesized that miRNAs bind to specific as-yet unidentified membrane receptors that are present on the recipient cells[33]. Each step of miRNA generation and function, both intracellular (FIG. 1) and endocrine (FIG. 2), can potentially be therapeutically targeted.

miRNAs and their roles in cancer

miRNAs have a variety of roles in cancer development and progression (BOX 3; TABLE 1). The involvement of miRNAs in cancer was first discovered in a quest to identify tumour suppressors in the frequently deleted 13q14 region in chronic lymphocytic leukaemia (CLL): the miRNA cluster miR-15a–miR-16-1 was found to be deleted or downregulated via epigenetic silencing in 69% of patients analysed[3]. Subsequent studies confirmed the tumour suppressor function of miR-15a–miR-16-1 and identified several other suppressor miRNAs such as the let-7 family and miR-34 family[5]. These tumour suppressor miRNAs exert their function by targeting oncogenic protein-coding genes for degradation[34]. For instance, the miR-15a–miR-16-1 cluster targets the genes coding for the anti-apoptotic proteins B cell lymphoma 2 (BCL-2) and induced myeloid leukaemia cell differentiation protein (MCL1); let-7 negatively regulates the oncogenes *KRAS* and *MYC*; and miR-34 mediates p53 signalling by targeting cyclin-dependent kinase 4 *(CDK4)*, *MYC* and *MET*[35] (TABLE 1).

miRNA cluster
A group of at least two microRNAs located close together on the genome (usually several hundreds of bases apart) that are generally transcribed in a unique transcript and also commonly regulated.

Table 1 | **Examples of the involvement of ncRNAs in cancer***

ncRNA	Type (genomic location)	Cancer involvement	Mechanism of action (target genes)	Refs
miR-10b	miRNA (chromosome 2)	Breast cancer	Promotes breast cancer metastasis (*HOXD10*)	158
miR-15a–miR-16-1	miRNA cluster (chromosome 13)	Downregulated in chronic lymphocytic leukaemia, diffuse large B cell lymphoma, multiple myeloma as well as prostate and pancreatic cancers	Induces apoptosis in leukaemia cells and regulates the cell cycle (*BCL2*, *CCND1*, *CDK6*, *DMTF1*, *MCL1*, *VEGF* and *TP53*)	3,54,159
miR-17–miR-92	miRNA cluster (chromosome 13)	Overexpression in lung and colon cancer, lymphoma, multiple myeloma and medulloblastoma	Increases tumour growth and tumour vascularization (*BIM*, *CDKN1A*, *E2F1*, *E2F2*, *E2F3*, *HIF1A*, *PTEN* and *TGFBR2*)	7,160,161
miR-21	miRNA (chromosome 17)	Overexpression in glioblastoma, breast, lung, prostate, colon, stomach, oesophageal and cervical cancers as well as diffuse large B cell lymphoma	Promotes invasion and metastasis in colorectal cancers; knockdown induces apoptosis in glioblastoma cells (*BCL2*, *SERPINB5*, *PDCD4*, *PTEN*, *TPM1* and *RECK*)	37,38,40, 162,163
miR-22	miRNA (chromosome 17)	Breast cancer	Regulates breast cancer stemness and metastasis (*TET*)	46
miR-31	miRNA (chromosome 9)	Breast cancer	Inhibits breast cancer metastasis (*RHOA*)	83
miR-34a, miR-34b, miR-34c	miRNA family (chromosomes 1 and 11)	Downregulated in pancreatic cancer, Burkitt's lymphoma without *MYC* translocation, $CD44^+$ prostate cancer and human primary breast tumours with lymph node metastases	Transcriptionally activated by p53; miR-34a inhibits prostate cancer stemness and metastasis; miR-34a, miR-34b and miR-34c suppress breast cancer cell invasion and metastasis (*BCL2*, *CCND1*, *CCNE2*, *CDK4*, *MYC*, *MYCN*, *MET*, *HMGA2*, *SIRT1*, *CD44* and *FRA1*)	164–168
miR-155	miRNA (chromosome 21)	Overexpression in paediatric Burkitt's lymphoma, Hodgkin's lymphoma, diffuse large B cell lymphoma as well as in breast, lung, colon and pancreatic cancers	Pre-B cell proliferation and lymphoblastic leukaemia or high-grade lymphoma in miR-155 transgenic mice (*AID* and *TP53INP1*)	42,169
miR-335	miRNA (chromosome 7)	Breast cancers	Inhibits breast cancer metastasis (*SOX4* and *TNC*)	170
miR-373, miR-520c	miRNA (chromosome 19)	Breast cancer	Promote migration and invasion of breast cancer cells *in vitro* and *in vivo* (*CD44*)	171
let-7 family	miRNA family (multiple locations)	Suppressor: downregulated in lung, breast, gastric, ovarian, prostate and colon cancers as well as in chronic lymphocytic leukaemia	Suppressor: represses cell proliferation and growth (*CCND1*, *CDK6*, *HOXA9*, *MYC*, *RAS* and *TLR4*)	172–174
		Oncogenic: overexpressed in acute myeloid leukaemia	Oncogenic: let-7a represses *NF2* and decreases chemotherapy-induced apoptosis *in vitro* (*CASP3*)	
BCYRN1	lncRNA (chromosome 2)	Multiple cancers	Protein binding	175,176
HOTAIR	lncRNA (chromosome 12)	Multiple cancers; promotes breast cancer metastasis	Reprogrammes chromatin state via epigenetic regulation (*HOXD10*)	108,109
MALAT1	lncRNA (chromosome 11)	Multiple cancers	RNA splicing, small RNA production, protein interaction	115,177, 178
PCA3	lncRNA (chromosome 9)	Upregulated in prostate cancer	Modulates androgen receptor signalling	179
PTENP1	Pseudogene (chromosome 9)	Downregulated in prostate cancer	Increases *PTEN* expression via miRNA decoy	117

AID, activation-induced cytidine deaminase; *BCL2*, B cell lymphoma 2; *BCYRN1*, brain cytoplasmic RNA 1 (also known as *BC200*); *BIM*, BCL-2-interacting mediator of cell death; *CASP3*, caspase 3; *CCND1*, cyclin D1; *CCNE2*, cyclin E2; *CDK*, cyclin-dependent kinase; *CDKN1A*, cyclin-dependent kinase inhibitor 1A; *DMTF1*, cyclin D-binding MYB-like transcription factor 1; *E2F1*, E2F transcription factor 1; *FRA1*, FOS-related antigen 1; *HIF1A*, hypoxia-inducible factor 1α; *HMGA2*, high-mobility group protein AT-hook 2; *HOTAIR*, HOX transcript antisense RNA; *HOXA9*, homeobox A9; *HOXD10*, homeobox D10; lncRNA, long non-coding RNA; *MALAT1*, metastasis-associated lung adenocarcinoma transcript 1; *SERPINB5*, serine peptidase inhibitor B5; *MCL1*, induced myeloid leukaemia cell differentiation protein; miRNA, microRNA; *MYCN*, neuroblastoma-derived MYC oncogene; ncRNA, non-coding RNA; *NF2*, neurofibromin 2; *PCA3*, prostate cancer antigen 3; *PDCD4*, programmed cell death protein 4; *PTEN*, phosphatase and tensin homolog; *PTENP1*, PTEN pseudogene 1; *RECK*, reversion-inducing cysteine-rich protein with kazal motifs; *SIRT1*, sirtuin 1; *SOX4*, transcription factor SOX4; *TET*, methylcytosine dioxygenase TET; *TGFBR2*, transforming growth factor-β receptor 2; *TLR4*, Toll-like receptor 4; *TNC*, tenascin C; *TP53*, tumour suppressor p53; *TP53INP1*, tumour protein p53 inducible nuclear protein 1; *TPM1*, tropomyosin 1; *VEGF*, vascular endothelial growth factor. *This table focuses on a few examples of non-coding RNAs described in this Review. For more detail, see REF. 34 for microRNAs and REF. 12 for long non-coding RNAs.

Box 2 | **Novel insights into miRNA properties**

The discovery that microRNAs (miRNAs) target the 3′ untranslated region (3′ UTR) of genes and downregulate the expression of protein-coding genes in the cytoplasm has been substantially expanded in the past 6 years with additional discoveries that have demonstrated unexpected complexities in the mechanism of action of miRNAs. Some of these discoveries are described below.

miRNA localization. miRNAs can be localized in the nucleus. For example, human miR-29b has been shown to predominantly localize to the nucleus[143]. It will therefore be more challenging to target nuclear miRNAs with anti-miRNA strategies.

'Non-classical' targets. In addition to 3′ UTRs, miRNAs target other genetic regions at the DNA level (promoter regions) or RNA level (5′ UTR; coding regions)[22,23,144,145]. They also target other non-coding RNAs such as transcribed ultraconserved regions (T-UCRs)[106] and even proteins[25]. For instance, miR-122 facilitates hepatitis C virus (HCV) RNA replication by binding directly to two adjacent sites close to the 5′ end of HCV RNA[76]. miRNAs can thus have much larger than anticipated effects on the whole transcriptome, as the spectrum of targets is much wider than that of coding genes.

Upregulation of protein translation. miRNAs can not only downregulate but also upregulate translation by diverse mechanisms. For example, miR-369-3 was shown to interact with AU-rich elements in tumour necrosis factor (*TNF*) mRNA and recruit the protein complex composed of Argonaute 2 (AGO2) and fragile X mental retardation-related protein 1 (FXR1) to the *TNF* mRNA, leading to increased protein translation during cell cycle arrest[24]. In the same study, the condition of cell cycle arrest switched the regulation of the miRNA let-7 on targeted genes from translational repression to translational activation. It has also been shown that miR-10a interacts with the 5′ UTR of ribosome protein-encoding mRNAs to enhance ribosomal biogenesis, which induces global protein synthesis and causes oncogenic transformation of murine NIH3T3 cells[26]. In another study, miR-328 was shown to increase the translation of the myeloid-specific transcription factor CCAAT/enhancer binding protein alpha (CEBPA) in chronic myelogenous leukaemia cells, not by directly binding to *CEBPA* mRNA but by directly binding to poly(rC) binding protein 2 (PCBP2), which interacts with a C-rich element located in the 5′ UTR of *CEBPA* mRNA and inhibits its translation[25]. However, it remains to be determined whether this activation of protein translation represents a general phenomenon or just exceptions of miRNA-regulatory mechanisms.

Competing endogenous RNA regulatory network. miRNAs interact with other non-coding RNAs and various types of mRNA transcripts in a 'competing endogenous RNA' (ceRNA) network[116]. Two co-expressed transcripts that are targeted by the same collection of miRNAs are functionally coupled to one another as a result of the finite amount of available miRNA: a transient change in the expression levels of one transcript will have a direct impact on the apparent abundance of the other transcript as a result of the concomitant change in the amount of miRNA that is available.

Exosomal miRNAs. miRNAs can also be packaged into multivesicular bodies and released into the extracellular environment as exosomes. This allows them to act as hormones, which are defined as secreted molecules that trigger a receptor-mediated response in a different cell or tissue[28,33,146]. It has been shown that macrophages influenced breast cancer cell invasion through the exosome-mediated delivery of oncogenic miR-223 (REF. 147), and pre-treatment of mice with tumour-derived exosomes accelerates lung metastasis formation[148]. Therefore, targeting miRNAs that are secreted by a specific cell could have an impact on a different cell type.

Toll-like receptor agonists. miRNAs can act as agonists of Toll-like receptors (TLRs) by interacting with *Tlr7* and *TLR8*, which triggers downstream pathway activation[27,92]. Therefore, modulation of miRNAs (for example, miR-29a) might not only lead to variations in target mRNA expression (for example, DNA methyltransferases)[149] but also induce changes in TLR-mediated signalling (for example, the nuclear factor-κB pathway, which is triggered by TLR8 activation).

Three years after miRNAs were first identified as tumours suppressors, several miRNAs — including miR-21, the miR-17–miR-92 cluster and miR-155 — were revealed to exhibit oncogenic activity in carcinogenesis. One of the most well-characterized oncogenic miRNAs is miR-21, which has been found to be upregulated in all types of cancer so far analysed[36]. Several studies have demonstrated the oncogenic functions of miR-21; for example, it has been shown that miR-21 overexpression induces pre-B cell lymphoma in mice[37], promotes KRAS-dependent lung carcinogenesis by activating the RAS–MEK (MAPK/ERK kinase)–ERK (extracellular signal-regulated kinase) pathway[38] and enhances metastasis of colorectal cancers by targeting programmed cell death protein 4 (PDCD4)[39]. Similarly, the miR-17–miR-92 cluster located at 13q22 is frequently upregulated in a broad range of cancer types, including lymphoma, lung cancer, breast cancer, stomach cancer, colon cancer and pancreatic cancer, through amplification or transcriptional activation[36]. miRNAs from this cluster are direct downstream targets of the oncogene *MYC* and they attenuate *MYC*-induced apoptosis to promote the formation of B cell lymphoma in mice[40,41]. The concept of miRNAs as an oncogenic driving force of cancer was demonstrated by showing that overexpression of miR-155 alone is sufficient to cause lymphoblastic leukaemia or high-grade lymphoma in transgenic mice[42].

It has been further demonstrated that some miRNAs can act as specific activators or suppressors of tumour metastasis[43]. Functional studies in animal models have shown that miR-9, miR-10b, miR-103, miR-107, miR-373 and miR-520c are drivers or promoters of metastasis, whereas miR-31, miR-34a, miR-126, miR-200, miR-206 and miR-335 suppress metastasis through diverse

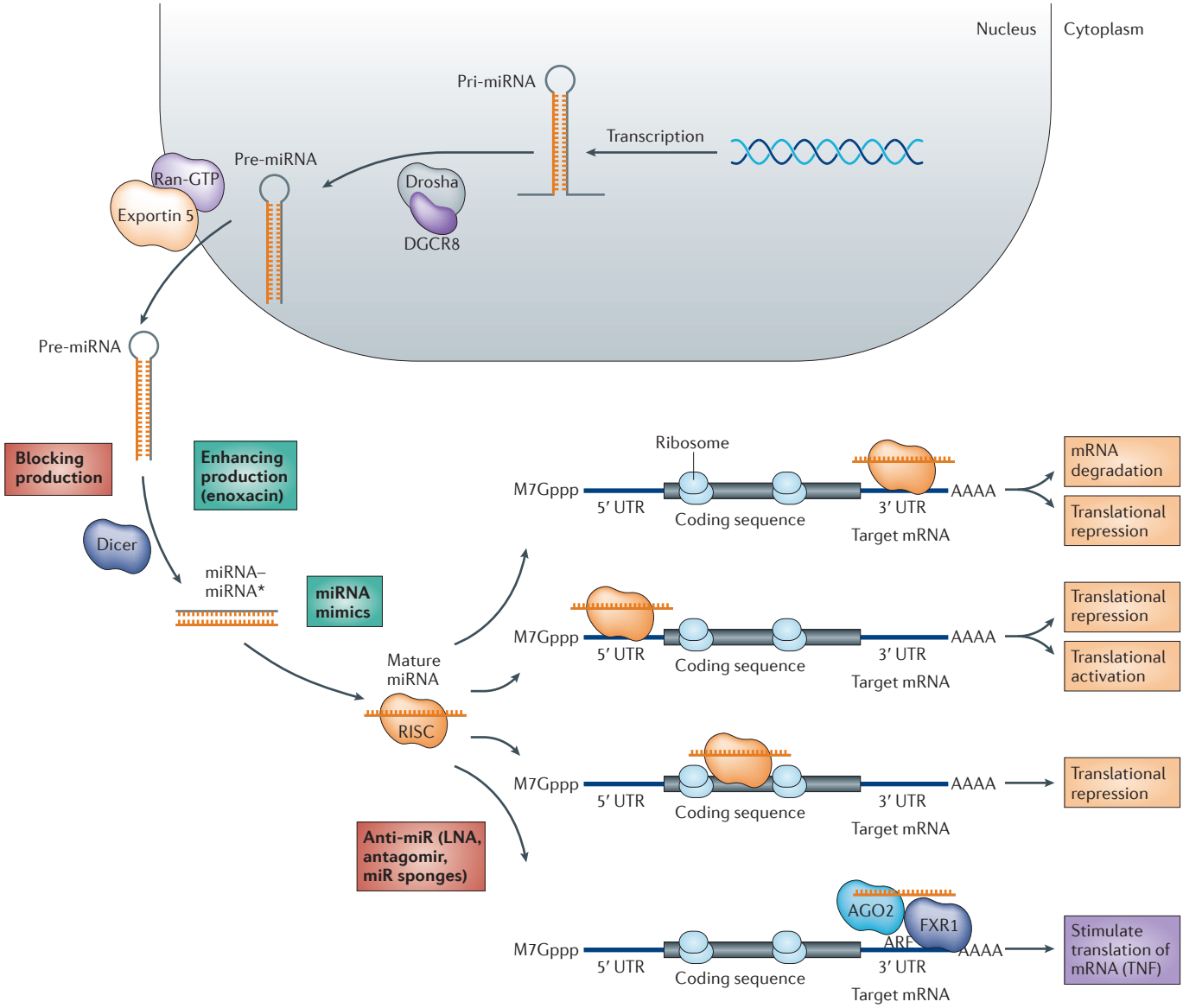

Figure 1 | **Mechanisms of action of miRNAs and the use of therapeutic agents to block or activate their function.** The diverse mechanisms of microRNA (miRNA) activity are presented together with the related miRNA-targeting strategies. The strategies that reduce the miRNA activity are shown in red boxes, whereas the strategies that increase miRNA activity are shown in green boxes. miRNA is initially transcribed as primary miRNA (pri-miRNA), then processed into precursor miRNA (pre-miRNA) by a microprocessor complex composed of Drosha and DiGeorge syndrome critical region 8 (DGCR8), and is then transported from the nucleus to the cytoplasm by exportin 5 in the presence of the Ran-GTP cofactor and further processed into its mature form by Dicer. The miRNA is then recruited to the RNA-induced silencing complex (RISC) and regulates the output of protein-coding genes through diverse mechanisms. The interaction of miRNAs with the 3′ untranslated region (3′ UTR) of protein-coding genes is considered as the main mechanism, which usually leads to a decrease in protein output either by mRNA degradation or by translational repression. Recent studies have also suggested that miRNAs can interact with the 5′ UTR of protein-coding genes via complementarity and cause translational repression[23] or activation of the targeted proteins[26]. Similarly, miRNAs can also target the coding sequence and repress the translation of targeted genes[22]. Moreover, some miRNAs can interact with regulatory protein complexes, such as Argonaute 2 (AGO2) and fragile X mental retardation-related protein 1 (FXR1), and indirectly upregulate the translation of a target gene[24]. It remains to be determined whether the 'non-canonical' mechanisms represent general mechanisms or exceptions. Various approaches can be used to enhance (for example, with enoxacin) or block (by targeting the biogenesis machinery) general miRNA production, but these approaches are not specific. More specific regulation of miRNA activity can be achieved using miRNA mimics or anti-miRs such as locked nucleic acids (LNAs), antagomirs and miR sponges, which bind and thereby functionally block specific miRNAs. Although most miRNA therapeutics are still in preclinical development, two have reached clinical trials: one LNA anti-miR (miravirsen) and one miRNA mimic (MRX34). ARE, AU-rich element; TNF, tumour necrosis factor.

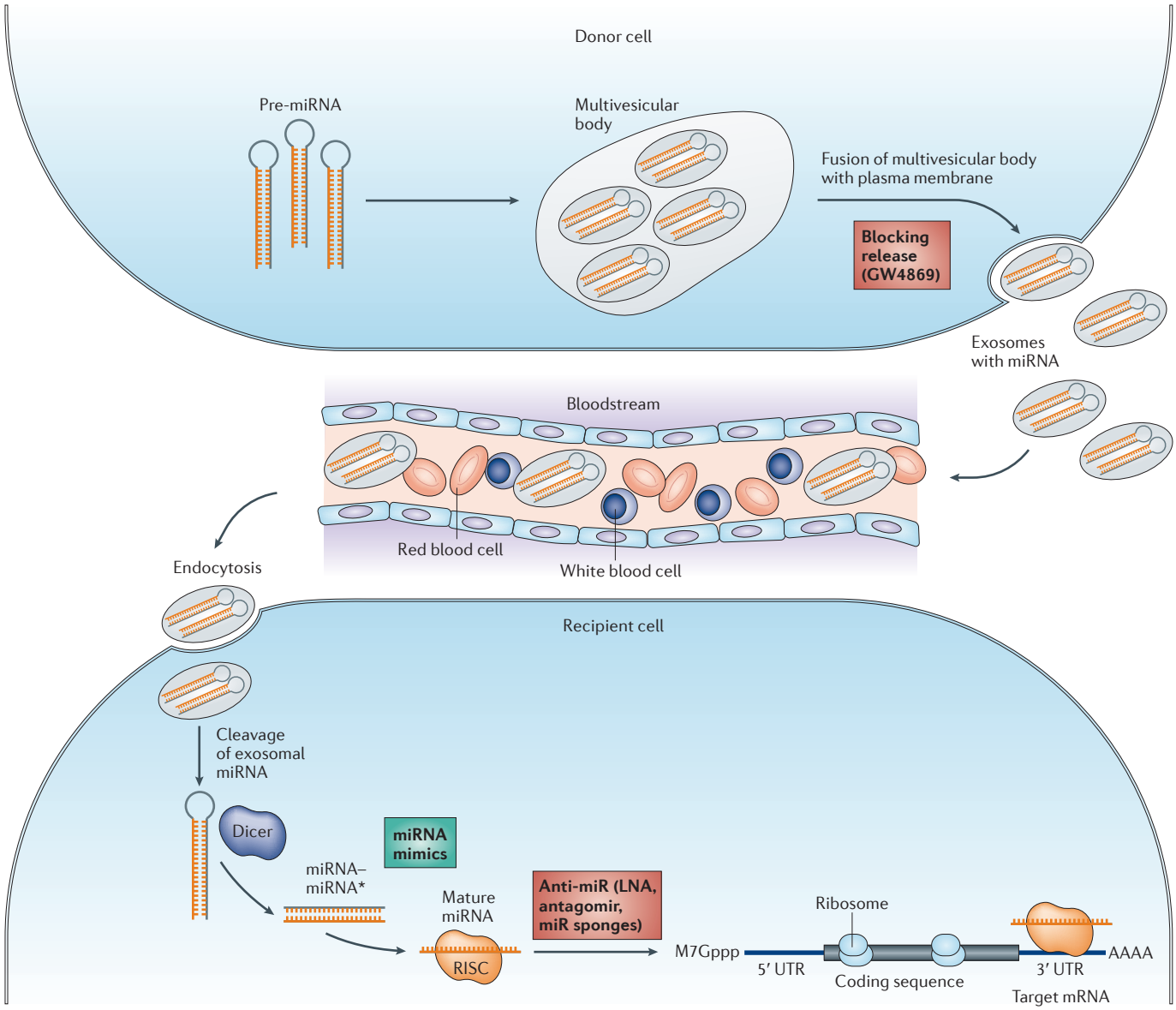

Figure 2 | **Interfering with miRNA exocrine function.** MicroRNAs (miRNAs) can also be packaged into multivesicular bodies, released into the extracellular environment as exosomes and carried through the circulatory system to act on recipient cells. This has been found to have a role in cancer development (BOX 2). Blocking of such secreted miRNAs can be achieved by interfering with their secretion from the cells of origin (such as cancer cells): for example, with inhibitors of neutral sphingomyelinase such as GW4869 (REF. 27). Alternatively, anti-miR strategies can be used to interfere with the function of secreted miRNAs in the recipient cell. Conversely, miRNA mimics with suitable formulation (for example, with lipid encapsulation) of secreted miRNAs can be used to enhance their function. The agents that reduce the miRNA activity are shown in red boxes, whereas the agents that increase miRNA activity are shown in a green box. 3′ UTR, 3′ untranslated region; RISC, RNA-induced silencing complex.

mechanisms[44]. Although metastasis-related miRNAs generally act by regulating the migration and invasion of cancer cells, miR-126 has been shown to impair the ability of breast cancer cells to recruit endothelial cells in the tumour microenvironment into the metastatic niche, thus inhibiting metastatic colonization[45]. A recent study revealed an interesting interaction between two miRNAs by showing that miR-22 increases the mammary stem cell population and promotes breast cancer metastasis in transgenic mice by reducing the expression of miR-200, which has an anti-metastatic function, via direct targeting of the methylcytosine dioxygenase TET, an enzyme that is responsible for the demethylation of the miR-200 promoter[46].

Complicating the issue of the involvement of miRNAs in cancer and their therapeutic targeting, it has been found that some miRNAs can behave like oncogenes in one cell type and as tumour suppressors in others. For example,

Box 3 | **The involvement of ncRNAs in cancer**

ncRNAs as oncogenes or tumour suppressors. Non-coding RNAs (ncRNAs) such as microRNAs (miRNAs) and transcribed ultraconserved regions (T-UCRs) are frequently located within cancer-associated genomic regions and can act as tumour suppressors or oncogenes[150]. Studies in several mouse models have strongly indicated that alterations in miRNA expression alone cause a cell to become neoplastic: miR-155-transgenic mice and miR-21-transgenic mice develop acute lymphoblastic or high-grade lymphoma[37,42], whereas mice in which the tumour suppressor cluster miR-15–miR-16 is functionally deleted develop chronic lymphocytic leukaemia[151].

ncRNAs in cancer metastasis. ncRNAs such as miRNAs and lncRNAs can also act as activators or suppressors of tumour metastasis. This is exemplified by miR-10b[81], which was shown to promote distant metastasis of breast cancer to the lung by negatively regulating the expression of homeobox D10 (*HOXD10*) and thus increasing the expression of the pro-metastatic gene *RHOC* and miR-31 (REF. 83), which suppressed breast cancer cell invasion and metastasis by targeting α5 integrin (*ITGA5*), radixin (*RDX*) and *RHOA*. lncRNAs have also been linked to tumour metastasis, as exemplified by the involvement of HOX transcript antisense RNA (*HOTAIR*) in breast cancer metastasis[108].

ncRNAs as diagnostic tools. Cancer cells are characterized by aberrant ncRNA gene expression signatures (such as miRNAs, T-UCRs and long intergenic ncRNAs (lincRNAs)), and ncRNA profiling can be used for cancer diagnosis, for establishing a prognosis and for determining the responsiveness of cancer patients to treatment. ncRNA profiles allow accurate and specific differentiation between tissue and disease types, so ncRNAs are of interest as biomarkers for early diagnosis and prognosis[9,12]. In particular, circulating miRNAs have high translational potential as non-invasive cancer biomarkers[28].

ncRNA regulatory networks. Each ncRNA (including miRNAs and long non-coding RNAs (lncRNAs)) can regulate the expression of numerous target genes; conversely, several types of ncRNAs can regulate the same target gene. The interplay among miRNAs, lncRNAs (such as lincRNAs, T-UCRs and pseudogenes) and protein-coding genes forms a complex network of interactions in normal tissues and this is dysregulated in many diseases, including all types of human cancer analysed so far[4,8,12].

Genetic variations affecting miRNA function. Variations in miRNA (and potentially also lncRNA) genes and their precursors, target sites and genes encoding components of the miRNA processing machinery (such as exportin 5 or TAR RNA binding protein 2 for miRNA processing) can affect the cell phenotype and disease susceptibility[152,153].

Epigenetic regulation by miRNAs. A subclass of miRNAs, known as epi-miRNAs, can directly control the epigenetic machinery (for example, by directly targeting DNA methyltransferases as in the case of miR-29 family)[149]. Conversely, miRNA expression can be downregulated via promoter hypermethylation[154].

when miR-221 is overexpressed in liver cancer, it exerts an oncogenic function by downregulating the expression of the tumour suppressor phosphatase and tensin homolog (PTEN)[47], but in erythroblastic leukaemia it acts as a tumour suppressor by reducing the expression of the *KIT* oncogene[48]. The same bivalent effects were also identified for the let-7 family (TABLE 1).

One paradigm that has emerged is the existence of miRNA–transcription factor feedback circuitries composed of multiple miRNAs and protein-coding genes, which can be involved in the pathogenesis of cancer. An example is the miRNA–*TP53* circuitry in CLL that is composed of five miRNAs (miR-15a–miR-16-1, miR-34a, miR-34b and miR-34c) and four coding genes including the transcription factor *TP53*, the 70-kDa ζ-associated protein *ZAP70* and the anti-apoptotic oncogenes *BCL2* and *MCL1*. The expression levels of the gene members of this circuitry were demonstrated to be powerful predictors of the survival of patients with CLL[49]. In acute T cell lymphoblastic leukaemia, a small set of miRNAs composed of miR-19b, miR-20a, miR-26a, miR-92 and miR-223 were found to cooperatively downregulate tumour suppressor genes including the DNA-binding protein IKAROS, PTEN, BCL-2-interacting mediator of cell death (BIM), PHD finger protein 6 (PHF6), neurofibromin 1 (NF1) and F-box and WD repeat domain-containing protein 7 (FBXW7), and to promote leukaemia development in a Notch 1-driven mouse model of leukaemia[50]. Similarly, the miR-17–miR-92 cluster and the protein-coding genes *MYC* and E2F transcription factor 1 (*E2F1*; also known as *RBBP3*) form a network that is involved in the development of lymphoma[51]. The complex interaction of miRNAs and protein-coding genes in cancer indicates that treatments targeting only protein-coding genes may not be sufficient for controlling cancer progression.

Considerations for miRNA therapeutics

As cancer is a multigenic disease, the main advantage of miRNA therapeutics (which are defined as strategies to restore or inhibit miRNA function) is that miRNAs target several coding genes or non-coding genes that can be involved in a specific pathway or in redundant pathways involved in cancer development. In other words, the ability of miRNAs to target genes that are implicated in the same pathway and/or in interacting pathways provides the rationale for the use of a small number of miRNAs to achieve an orchestrated broad silencing of pro-tumoural pathways; for this reason, miRNA therapeutics may be superior to a mixture of small interfering RNAs (siRNAs) that are specifically designed to reduce the expression of a given number of

target genes. For instance, miR-124 has been shown to target several protein-coding genes such as p38 mitogen-activated protein kinase (p38 MAPK), signal transducer and activator of transcription 3 (STAT3) and AKT2 in the epidermal growth factor receptor (EGFR) signalling pathway[52]. As constitutive activation of EGFR signalling has been linked to multiple cancer types, including lung, colon and breast cancers[53], it can be hypothesized that miR-124 replacement by mimics or vector-encoded miR-124 may simultaneously downregulate the expression of these three protein-coding genes and thereby reverse cancer progression by inactivating the EGFR signalling pathway.

The miR-15a–miR-16-1 cluster — which targets the anti-apoptotic proteins BCL-2 and MCL1 — has been found to be downregulated in CLL[54]. Therefore, a personalized miRNA therapy with agents that either mimic or boost the expression of miR-15a and miR-16-1, or a therapy with vector-encoded miR-15a and miR-16-1, can be envisioned for patients with CLL who have both reduced expression of the miR-15a–miR-16-1 cluster and overexpression of BCL-2 and MCL1 in their malignant cells. miR-15a and miR-16-1 also have an inhibitory effect on other anti-apoptotic targets such as the DNA repair protein RAD51C, programmed cell death 6 interacting protein (PDCD6IP), 78 kDa glucose-regulated protein (GRP78; also known as HSPA5) and protein disulphide isomerase A2 (PDIA2)[54]. This multiple targeting is thought to have an additive effect in inhibiting anti-apoptotic signalling.

Mutations in miRNAs, although previously reported[55], are rare owing to the small size of miRNAs; furthermore, the development of resistance to miRNA therapeutics would probably require multiple mutations in several genes. Circulating miRNAs could be used as biomarkers to identify the patients for whom this approach would be suitable (see BOX 4 for a discussion of miRNAs as biomarkers).

However, there are also substantial challenges associated with miRNA-targeted approaches. As discussed above, miRNA activity can be dependent on the cellular environment, and the same miRNA can have different targets in the same organism (but in different cell types) and consequently opposite effects. Therefore, modulation of a specific miRNA with miRNA therapeutics might have beneficial effects in one cell type but harmful effects in another.

Further complicating the issue is the finding (reported for various miRNAs) that the mature products generated from each strand of the same hairpin RNA structure, termed 5p and 3p, can bind to different mRNAs and display bivalent behaviour. For example, miR-28-5p and miR-28-3p are transcribed from the same RNA hairpin and are downregulated in colorectal cancer cells[56]. By contrast, miR-28 is upregulated in some BCR–ABL-negative myeloproliferative neoplasms[57]. *In vitro* experiments with colorectal cancer cells showed that the overexpression of either miR-28-5p or miR-28-3p had different effects owing to the fact that the two miRNAs interacted with different mRNAs: miR-28-5p downregulated the expression of cyclin D1 (CCND1) and homeobox B3 (HOXB3) proteins, whereas miR-28-3p downregulated the expression of the metastasis suppressor protein NM23-H1 (also known as NDKA). Overexpression of miR-28-5p reduced colorectal cancer cell proliferation, migration and invasion *in vitro*, whereas miR-28-3p increased colorectal cancer cell migration and invasion *in vitro*[56]. Similarly, it has been reported that miR-125a-5p and miR-125a-3p, which are downregulated in non-small-cell lung cancer (NSCLC), exhibit distinct effects *in vitro* on the migration and invasion of lung cancer cells[58].

Such information has direct implications for the design of miRNA gene therapy trials: if a precursor miRNA inserted in a viral vector is to be used and both strands are produced, the identification of the specific roles of each strand in the same cell type is mandatory. If opposite effects are observed (such as for miR-28 or miR-125a), then such precursors should be excluded from use and the respective active mature miRNA should be either delivered as an miRNA mimic (for example, miR-28-5p or miR-125a-3p) or inhibited (for example, miR-28-3p or miR-125a-5p) (TABLE 2).

The development of miRNA therapeutics

miRNA therapeutics are being devised that downregulate or block the function of oncogenic miRNAs or that upregulate the expression of miRNAs that have a tumour-suppressive function (FIG. 1). The main causes of the reduced expression of tumour suppressor miRNAs in human cancers are genetic deletion of the miRNA loci or epigenetic silencing via CpG island hypermethylation in the promoter of the miRNA genes[11]. Molecular approaches are being pursued that reverse epigenetic silencing or enhance the biogenesis of miRNAs, and levels of silenced or deleted miRNAs can be restored by the direct administration of miRNA formulations — naked, coupled to a carrier or delivered via a viral vector. Likewise, for strategies that block miRNA functions, both oligonucleotide-based and small-molecule-based approaches are being explored. miRNAs can be modulated either intracellularly in the cells that produce them, or their endocrine function can be targeted (FIG. 2). Various approaches are discussed below.

Restoring miRNA levels with small molecules. Epigenetic silencing of miRNAs can be reversed by hypomethylating agents such as decitabine or 5-azacytidine. Both agents have been approved for the treatment of myelodysplastic syndromes[59] and have been shown to re-induce the expression of several mRNAs as well as ncRNAs, including miRNAs[60]. However, this is a nonspecific miRNA effect and the spectrum of induced miRNAs varies from cell to cell[61,62]. Another example is the small molecule enoxacin, a fluoroquinolone that is used as an antibacterial compound, which has been shown to enhance the production of a subset of miRNAs by binding to the miRNA biosynthesis protein TAR RNA-binding protein 2 (TARBP2)[63]. Treatment of RKO and HCT116 colon cancer cells with enoxacin caused an overall upregulation of miRNA expression *in vitro*. Enoxacin treatment increased the expression of 24 mature miRNAs in mice and reduced tumour growth in xenograft,

Box 4 | **Circulating miRNAs as non-invasive biomarkers**

The biomarker potential of microRNAs (miRNAs) in the body fluid relies mainly on their high stability and resistance to storage and handling. Expression patterns have been identified of serum miRNAs that are specific for lung cancer, colorectal cancer and diabetes, which indicates that serum miRNAs can provide fingerprints for various diseases[155]. Correlations between circulating miRNA levels and the response to a given anticancer agent have also been observed and may be useful in predicting patterns of resistance and sensitivity to particular drugs. For example, circulating miR-21 levels were higher in patients with hormone-refractory prostate cancer whose disease was resistant to docetaxel-based chemotherapy, in comparison with patients who had chemosensitive disease[156]. High levels of miR-141 expression in the plasma have been associated with poor prognosis in patients with colorectal cancers[157].

orthotopic and metastatic mouse models[63]. Interestingly, the drug's growth-inhibitory effect was substantially compromised both in a colon cancer cell line with an inactivating mutation in the *TARBP2* gene and in *in vivo* studies with TARBP2-deficient mice[63], which indicates that miRNA regulation by enoxacin is the main mechanism for its anticancer effect. These examples highlight the key role of disrupted miRNA expression patterns in cancer and demonstrate the effectiveness of fully restoring the distorted spectrum of miRNAs that are downregulated in cancer cells.

Restoring miRNA levels with oligonucleotide-based approaches. A more targeted approach for boosting the levels of particular miRNAs, compared to the strategies discussed above, is the restoration of the expression and function of one or a limited number of miRNAs, usually located in a cluster (such as miR-15a and miR-16-1 at 13q14.3), either with miRNA mimics or with miRNAs encoded in expression vectors.

miRNA mimic molecules are double-stranded synthetic miRNA oligonucleotides that, when transfected into cells, are processed into a single-strand form and regulate protein-coding genes in an miRNA-like manner. However, an effective delivery system is necessary to improve the stability and uptake — and thus the efficacy — of miRNA mimics[64]. One strategy is to couple miRNA mimics to nanoparticles coated with tumour-specific antibodies, as exemplified by a study showing that the targeted delivery of miR-34a using nanoparticles coated with a neuroblastoma-specific anti-disialoganglioside GD_2 antibody inhibited neuroblastoma tumour growth in a murine orthotopic model[65]. It was also shown that systemically delivered neutral lipid emulsions of miR-34a and let-7 mimics induced a significant reduction in tumour burden in a KRAS-activated mouse model of NSCLC[66]. Moreover, formulations with atelocollagen led to the efficient delivery of miR-34a into tumours and inhibited colon cancer progression in a subcutaneous xenograft mouse model[67].

miRNA expression vectors are engineered with promoters that enable the expression of the miRNA of interest in a tissue- or tumour-specific fashion. This approach has been demonstrated in hepatocellular carcinoma (HCC) both *in vitro* and in a xenograft mouse model. miR-26a expression is reduced in human primary HCC (with respect to the normal liver tissue counterpart) and its re-expression in hepatic malignant cells leads to anti-tumour activity[68]. Systemic administration of miR-26a in a mouse model of HCC using an adeno-associated virus (AAV) was shown to inhibit cancer cell proliferation, induce tumour-specific apoptosis and slow disease progression with minimal toxicity[69]. Interestingly, a recombinant miR-26a expression vector driven by a dual promoter for α-fetoprotein and human telomerase reverse transcriptase was shown to be specifically expressed in liver tumour cells and caused a reduction in HCC formation, at least in part by directly downregulating the expression of oestrogen receptor-α[70].

The various strategies for restoring miRNA function have yielded the first miRNA replacement therapeutic in the clinical pipeline: MRX34, an intravenously injected liposome-formulated miR-34 mimic with a diameter of ~120 nm, which is in clinical trials for patients with advanced or metastatic liver cancer. Preclinical studies have shown that tail vein injection of MRX34 reduced tumour growth and significantly enhanced survival, with a favourable safety profile, in orthotopic mouse models of hepatocellular carcinoma[71].

Blocking miRNA function with oligonucleotide-based approaches. Current strategies for inhibitory miRNA targeting (TABLE 2) are mainly based on antisense oligonucleotides (ASOs; also known as anti-miRs, which include locked nucleic acids (LNA anti-miRs), tiny LNA anti-miRs and antagomirs) and miRNA sponges.

LNA anti-miRs are ASOs in which several nucleotides are substituted by bicyclic RNA analogues in a 'locked' conformation (LNA molecules). These hold the highest affinity to the targeted miRNA as the 'locked' modification of the ribose ring — engineered through the addition of a methylene bridge connecting the 2′-oxygen atom and the 4′-carbon atom — creates an ideal conformation for Watson–Crick binding to the targeted miRNA. This allows effective blocking to be achieved with short sequences of 13-22 nucleotides, equalling the size of the targeted miRNA[72]. Several LNA anti-miR-based approaches are currently undergoing preclinical investigation for cancer. For instance, high expression of miR-380-5p was associated with a poor outcome in neuroblastoma with MYCN amplification, and the *in vivo* delivery of an LNA-anti-miR-380-5p efficiently reduced tumour size in an orthotopic mouse model of neuroblastoma, probably by reversing the suppression of p53 by miR-380-5p[73]. However, the agent that is furthest in development is not an anticancer compound but an antiviral one; miravirsen (SPC3649; Santaris Pharma), an LNA anti-miR against miR-122, was recently evaluated in Phase I and Phase IIa clinical trials for the treatment of hepatitis C virus (HCV)[74,75]. The rationale for these studies was based on the observation that liver-specific miR-122 binds to two miR-122 target sites in the 5′ non-coding region of the HCV genome, leading to an upregulation of viral RNA levels[76].

Tiny LNA anti-miRs are fully LNA-modified ASOs that are 8 nucleotides long and specifically designed to target the 5′-seed region (comprising 2–8 nucleotides) of

Table 2 | **The principal types of RNA therapeutic drugs**

Agent	Target RNA	Description of agent	Mechanism of action	Stage of development
Blockade				
Antisense oligonucleotides (ASOs)	All RNAs	ASOs are single-stranded, chemically modified DNA-like molecules (13–25 nucleotides in length) that are complementary to a selected RNA; LNA anti-miRs and antagomirs fall into this category	Formation of an RNA–ASO duplex through Watson–Crick binding, which leads to RNase H-mediated cleavage of the target RNA[180]; antagomirs silence miRNA in an as-yet unidentified way	Preclinical studies, Phase I and IIa for LNA anti-miRs
Small interfering RNAs (siRNAs)	All RNAs	siRNAs are double-stranded RNAs that are homologous to the target RNAs including mRNAs and non-coding RNAs, both of which are targeted based on perfect sequence complementarity	siRNAs are incorporated into a multiprotein RNA-induced silencing complex, leaving the antisense strand to guide the complex to its homologous mRNA or ncRNA target for the endonucleolytic cleavage of RNA[181]	Preclinical studies
LNA anti-miRs and tiny LNA anti-miRs	miRNAs	LNA anti-miRs are partially LNA-modified ASOs; LNAs are bicyclic RNA analogues with a ribose ring that is locked in a C3′-endo conformation by the introduction of a 2′-O,4′-C methylene bridge[79]; LNA anti-miRs are 13–22 nucleotides long; tiny LNA anti-miRs are 8 nucleotides long, are fully LNA-modified and are specifically designed to target the 5′ seed region of miRNAs	Formation of an RNA–ASO duplex through Watson–Crick binding, which leads to RNase H-mediated cleavage of the target RNA[180]	Phase I and IIa (for hepatitis C virus)[74,75]
Antagomirs	miRNAs	Antagomirs are single-stranded ~23-nucleotide-long RNA molecules that are complementary to the targeted miRNA and have been modified to increase the stability of the RNA and protect it from degradation; the modifications include a partial phosphorothioate backbone in addition to a 2′-*O*-methoxyethyl group[80]	Antagomirs silence miRNA in an as-yet unidentified way; the miRNA–antagomir–duplexes induce degradation of the miRNA and recycling of the antagomir[80]	Preclinical studies
miR sponges	miRNAs	miR sponges are RNAs that contain multiple tandem binding sites to an miRNA of interest and are transcribed from expression vectors[82]	By competing with the native targets of miRNAs, these highly expressed transcripts reduce the effects of miRNAs and thus result in increased expression of the miRNA's native targets[82]	Preclinical studies
Small-molecule drugs that target specific miRNAs (SMIRs)	miRNAs	SMIRs are small-molecule chemical compounds that interfere with miRNAs	Blockade of the activities of specific miRNAs by structure-based docking onto the precursor or mature form of the miRNA structure	Preclinical studies
Ribozymes and deoxyribozymes	lncRNAs	A ribozyme, or RNA enzyme, is an RNA molecule that can catalyse a chemical reaction; a deoxyribozyme is a catalytic DNA that cleaves target RNA in a site-specific manner	Three steps, cyclically repeated: Watson–Crick base pairing with a complementary target sequence, then site-specific cleavage of the substrate followed by the release of the cleavage products	Preclinical studies
Restoration				
Small molecules	miRNAs	Small-molecule RNA therapeutics include hypomethylating agents (such as decitabine or 5-azacytidine) and enoxacin	Nonspecific induction of miRNA expression	Preclinical studies
miRNA mimics	miRNAs	miRNA mimics are double-stranded synthetic RNAs that mimic endogenous miRNAs	Restoration of the expression and function of a specific miRNA	Phase I
miRNA expression vectors	miRNAs	miRNA expression vectors express a specific type of miRNA	Restoration of the expression and function of a specific miRNA	Preclinical studies

LNA, locked nucleic acid; lncRNA, long non-coding RNA; miRNA, microRNA; ncRNA, non-coding RNA.

miRNAs. They were shown to concurrently bind to and sequester miRNAs sharing the same seed sequence, and thereby increase the expression of miRNA-suppressed protein-coding genes[77]. Intravenous injection of unconjugated tiny LNA anti-miRs achieved long-term silencing of the targeted miRNAs with high specificity and efficacy in a murine orthotopic breast tumour model[77]. A concern associated with tiny LNA anti-miRs could be their off-target effects on mRNAs with perfect complementarity; however, comprehensive RNA and protein profiling showed that tiny LNA anti-miRs did not substantially alter the output of such protein-coding genes[77]. In another study, a tiny LNA anti-miR targeting miR-155 inhibited Waldenström macroglobulinaemia and CLL cell proliferation *in vitro*, and significantly decreased the number of leukaemic cells in a mouse model when

administered in an unconjugated form by tail vein injection[78]. However, it should be pointed out that although tiny LNA anti-miRs have the advantage of targeting multiple miRNAs from the same family, longer oligonucleotides are necessary if a more specific effect towards an individual miRNA is preferred. The high efficacy of LNA anti-miRs, their resistance to degradation and their efficient uptake by many tissues eliminate the need for sophisticated formulation and delivery, which is required for most other oligonucleotide treatments. Moreover, no acute or subchronic toxicities have been observed, and levels of plasma transaminases and bilirubin were not affected in primates treated with LNA anti-miRs[79].

Antagomirs are cholesterol-conjugated synthetic RNAs with a 2′-*O*-methyl linkage and phosphorothioate modification. They function as anti-miRs by being complementary to the full sequence of the targeted miRNA and thus blocking miRNA function[80] (TABLE 2). The cholesterol modification is introduced to increase the cellular uptake, whereas the 2′-*O*-methyl and phosphorothioate modifications are designed to improve the binding affinity and prevent degradation by nucleases, respectively. In a study investigating the role of miR-10b in metastasis using an orthotopic xenograft murine model of metastatic breast cancer, researchers silenced miR-10b with a specific antagomir via intravenous delivery[81]. They observed that silencing miR-10b did not reduce the growth of the primary tumour, but there was an impressive reduction in the formation of lung metastases. Interestingly, the effects of the antagomir were fully replicated using anti-miR-10b sponges (see below). However, when the lung nodules developed from disseminated cells that were directly injected into the tail vein, antagomir-10b had no effect on lung metastasis formation. These findings indicate that miR-10b is not involved in the late stage of metastasis formation. The toxicity of the antagomir was limited to a slight reduction (just below the normal range) in white blood cell count, an 8–9% increase in liver and spleen size, a two-fold increase in serum bilirubin (which was still in the normal range) and a slight increase in the levels of transaminases[81]. It should be noted that although antagomirs are routinely being used as experimental tools, most miRNA therapeutic agents in the developmental stage used other modifications such as 2′-fluoro substitutions or LNAs, largely because antagomirs require high dosages for effective miRNA blocking[72].

miRNA sponges are RNAs that are designed to contain multiple tandem binding sites that are complementary to a heptamer in the seed sequence of the miRNA of interest, and thus a single type of sponge can be used to block an entire miRNA seed family. As the efficacy of miRNA sponges also depends on the concentrations of sponge RNAs, they are usually encoded in either plasmid or viral expression vectors that are driven by a strong promoter such as the cytomegalovirus promoter, which can be stably transfected to allow for long-term delivery of the miRNA sponge after a single application[82]. miRNA sponges have been used to study tumour metastasis-related miRNAs, and using orthotopic mouse models it was found that sponges targeting miR-31 (REF. 83), miR-9 (REF. 84) or miR-10b[81] dramatically reduced the expression of the respective miRNAs and effectively enhanced (sponges targeting miR-31) or blocked (sponges targeting miR-9 or miR-10b) breast cancer metastases.

In another study, sponges targeted at the seed regions of the miR-17–miR-92 cluster simultaneously silenced each miRNA member: that is, miR-17, miR-18a, miR-19 and miR-92a[85]. Compared with miRNA sponges targeting individual miRNAs (for example, miR-92a alone), the combined targeting of the miR-17–miR-92 cluster had a stronger inhibitory effect on the proliferation of WEHI-231 BCL cells *in vitro*[85]. With regard to the stable transfection of vectors encoding miRNA sponges, it has been reported that recombinant AAV vectors encoding an anti-miR-122 sponge depleted levels of the corresponding miRNA in the liver of treated mice and reduced serum cholesterol levels by >30% for 25 weeks when compared with the control group[86]. Although this was not a cancer-related study, the long-lasting effect of miRNA sponges has an immediate application in cancer treatment as anticancer strategies often require persistent target regulation. However, it should be noted that as sponges use competitive RNAs without chemical modifications, their binding affinity is relatively low and the concentrations needed for effective miRNA blocking are likely to be much higher than with LNA anti-miRs or antagomirs. Moreover, it remains to be determined whether an excess of sponge transcripts that are not sequestered by the target miRNAs can have undesired effects.

The challenges associated with anti-miR treatment include off-target effects, which can lead to unwanted responses in tissues other than the targeted ones. Although modifications, such as the chemical substitutions of antagomirs and LNA anti-miRs as described above, can be combined and may improve the *in vivo* bioavailability, stability and specificity of the anti-miR towards a specific miRNA, a specific delivery system that targets only tumour cells has not yet been developed. Another obstacle for anti-miR therapeutics is the complexity of assessing their efficacy. This is because anti-miR treatment may not always reduce miRNA expression levels. For instance, miravirsen was shown to sequester miR-122 by forming a highly stable heteroduplex rather than by degrading the mature miR-122 (REF. 74). It remains to be determined under which conditions the anti-miR treatment will cause degradation of the targeted miRNAs, and this is partially complicated by the possible interference of the anti-miR–miRNA duplex when using detection methods such as PCR and northern blots[87]. In order to evaluate the efficacy of an anti-miR, it is necessary to profile not only the expression level of miRNAs in cancer cells but also the extent of target de-repression. miRNAs have multiple mRNA targets and, as suggested by a recent study, the miRNA-mediated modulation of protein-coding gene targets is a fine-tuning effect rather than an 'on–off' effect[88]. High-throughput profiling of global changes in mRNA and protein expression in samples could provide more comprehensive information regarding the specificity and effectiveness of a particular anti-miR treatment.

Small molecules targeting miRNAs. Although the bulk of research within the miRNA therapeutics field focuses on oligonucleotide-based approaches, there are several reports of efforts to identify small-molecule drugs that target specific miRNAs (SMIRs) and modulate their activities. For example, high-throughput screening of chemical compounds and structure–activity relationship analyses have identified diazobenzene and its derivatives as effective inhibitors of pri-miR-21 formation, but the sequence specificity was not comprehensively tested[89]. Small-molecule inhibitors of the liver-specific miRNA miR-122 (such as 2,4-dichloro-*N*-naphthalen-2-ylbenzamide and 6-[(4ar,8as)-octahydroquinolin-1(2h)-ylsulphonyl]-1,2,3,4-tetrahydroquinoline) have also been identified in a reporter plasmid assay and their specificity towards miR-122 was demonstrated[90]. Despite these findings, the underlying mechanisms of action of SMIRs are not clear. The structural features of miRNAs — such as the stem loops in pre-miRNAs — may partially uncover the internal bases, scattering the local electronegative distribution, and thus facilitate the binding of a SMIR to the grooves and pockets on the surfaces of a specific miRNA[91]. The advantages of SMIRs are that they are chemical compounds and thus conventional drug development can be applied. The limitations of SMIRs are their intrinsic poor specificity, possible unwanted miRNA-independent effects and their more complicated design compared with oligonucleotide-based therapeutics.

Blocking extracellular miRNAs in exosomes. As described above, it was recently discovered that miRNAs (such as miR-21, miR-29a and miR-16) can be released by cancer cells within exosomes. When engulfed by macrophages in the tumour microenvironment, the miRNAs colocalize with TLR8 in the endosome of the recipient cells and activate the single-stranded RNA-specific *TLR8* in humans (or its murine homologue *Tlr7*). This leads to increased secretion of interleukin-6 (IL-6) and tumour necrosis factor (TNF) by the immune cells, which can increase the proliferation and metastatic potential of cancer cells[27]. It remains to be determined whether the endogenous miRNAs can have similar effects on TLR8, and whether this miRNA effect is sequence-specific.

It has also been shown that extracellular let-7 (which is a highly abundant regulator of gene expression in the central nervous system) can activate *Tlr7* expression in mice and induce neurodegeneration through neuronal *Tlr7* signalling[92]. Intriguingly, let-7b levels are higher in the cerebrospinal fluid of patients with Alzheimer's disease, which indicates that miRNA-mediated activation of TLRs could have implications beyond cancer[92]. This novel mechanism of action and its potential central role in cancer dissemination and neurodegenerative diseases harbours important translational implications. It has also been demonstrated that ceramide-dependent secretion is one of the mechanisms leading to the release of exosomic miRNAs, and the small molecule GW4869 — an inhibitor of neutral sphingomyelinase that has a key role in ceramide biosynthesis — effectively blocked the miRNA-mediated aberrant crosstalk between cancer cells and surrounding immune cells within the tumour microenvironment in NSCLC[27].

Combination approaches

The concept of combinations of various miRNA agents in 'cocktails', together with chemotherapeutic agents or molecularly targeted agents, might benefit patients by resulting in synergistic effects. Encouraged by positive results from the Phase II clinical trial of miravirsen in controlling HCV infection, a Phase II clinical trial that tested the combination of this anti-miR with clinically used antiviral drugs such as telaprevir and ribavirin was initiated and is now recruiting participants (ClinicalTrials.gov identifier: NCT01872936).

For a cancer such as CLL, two combination strategies for the inhibition of RNA expression can be envisioned. The 'sandwich RNA inhibition strategy' would focus on a major molecular alteration that is clearly linked to CLL pathogenesis through the use of multiple agents. For example, clinical studies in relapsed or refractory CLL have shown variable efficacy of oblimersen sodium, an antisense oligonucleotide designed to specifically bind to human *BCL2* mRNA[93]; in some studies a significant increase in overall survival was observed, whereas in others there was no observed advantage. It has been suggested that the fluctuation in oblimersen's efficacy is related to difficulties in drug delivery to the unfavourable tumour environment[94]. Owing to the physiological presence of miRNAs in most tissues and the finding that miRNAs are very stable in body fluids[33], strategies using miRNA mimics may bypass the lower efficiency due to delivery obstacles. Thus regimens using a cocktail of antisense oligonucleotides (such as oblimersen) or siRNAs against BCL-2 and miRNAs targeting BCL-2 (which is specifically overexpressed in malignant B cells but not in surrounding non-malignant blood, lymph node or bone marrow cells), such as miR-15a and miR-16, might conceivably achieve a better therapeutic outcome for indolent CLL. Two *in vitro* studies have recently shown that combined treatment with BCL-2 siRNA and miR-15a synergistically enhanced methotrexate-induced apoptosis *in vitro* in Raji cells[95,96].

Another strategy, which we call the 'multiplex RNA inhibition strategy', could target various molecular defects in the same pathway. For instance, for CLL, strategies to restore the function of miR-15a and miR-34a may achieve a better effect in inducing the apoptotic response by reducing the expression of anti-apoptotic proteins such as MCL1 (by miR-15a), sirtuin 1 (by miR-34a) and BCL-2 (by miR-15a and miR-34a).

Sensitization of tumours to therapy via combined use of miRNA and chemotherapeutic agents. Glucocorticoids are effective agents in the treatment of lymphoid malignancies, and resistance to glucocorticoid-induced apoptosis is associated with poor prognosis of mixed lineage leukaemia (MLL)–AF4-driven acute lymphocytic leukaemia (ALL)[97]. In MLL–AF4 ALL cells, downregulation of miR-128b and miR-221 is implicated in glucocorticoid resistance[98]. It was demonstrated that miR-128b

negatively regulates the expression of oncogenes such as *MLL* and *AF4* as well as the fusion genes *MLL–AF4* and *AF4–MLL*, and a mutation in the miR-128b gene was shown to lead to glucocorticoid resistance owing to reduced production of mature miR-128b[99]. Thus the restoration of miR-128b levels could potentially revert glucocorticoid resistance, and combined treatment with anti-miR-128b and glucocorticoids could be envisioned.

In another study, ectopic expression of miR-30c, which is a good prognostic marker for human breast cancer, was shown to sensitize cancer cells to doxorubicin treatment in a xenograft mouse model of triple-negative human breast tumours. This sensitization was achieved by the negative regulation of twinfilin 1 (*TWF1*) and *IL11*, two protein-coding genes that regulate drug sensitivity[100]. Similarly, the combination of 5-fluorouracil with an adenoviral vector expressing the tumour suppressor miR-145 was shown to exert a stronger antiproliferative effect than 5-fluorouracil treatment alone, in both *in vitro* and *in vivo* models of breast cancer[101]. In another study, an LNA–anti-miR-21 combination was shown to increase the efficacy of a secreted variant of the cytotoxic agent TNF-related apoptosis-inducing ligand (S-TRAIL) in the treatment of glioma. The LNA–anti-miR-21 combination enhanced S-TRAIL-induced caspase activation and thereby the apoptotic response, and the combination led to complete eradication of gliomas in a murine xenograft model with human glioma U87 cells[102].

lncRNAs in cancer and therapeutic implications

ncRNAs other than miRNAs might also hold potential for new anticancer approaches[21]. lncRNAs are excellent candidates in this respect, and several lncRNAs have been found to be abnormally expressed in human cancers and to participate in cancer pathogenesis. lncRNAs are defined as RNA molecules that are longer than 200 nucleotides (BOX 1; BOX 3) and are not translated into proteins. Based on their structural or functional characteristics, they can be further separated into multiple subgroups such as circular RNAs (circRNAs)[103], natural antisense transcripts (NATs)[104,105], transcribed ultraconserved regions (T-UCRs)[106], long enhancer ncRNAs[107], long intergenic ncRNAs (lincRNAs) and pseudogenes. Recent studies indicate that lncRNAs are predominately located in the nucleus, but their functional mechanisms have not yet been well elucidated, and members of the same subfamilies of lncRNAs may display distinct mechanisms of action.

lincRNAs, which are transcribed from DNA sequences between protein-coding genes, were identified using histone marker signatures associated with RNA polymerase II, specifically by the trimethylation of lysine 4 and lysine 36 of histone 3 (K4K36)[108,109]. About 20% of lincRNAs regulate the transcriptional activity of protein-coding genes by guiding the histone methyltransferase Polycomb repressive complex 2 (PRC2) to specific genomic loci[110,111]. To date, one of the best studied lincRNAs is HOX transcript antisense RNA (*HOTAIR*), a 2.2 kb lincRNA residing in the *HOXC* locus, which was found to be highly expressed in breast cancer samples[108]. *HOTAIR* was shown to redirect PRC2 to specific genomic loci via direct interaction with PRC2 and thus cause downregulation of a specific set of genes, thereby regulating cancer invasion and metastasis[108,109]. Another lincRNA, metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*), was found to predict metastasis and survival in early-stage NSCLC[112]. It was also shown that many p53-regulated lincRNAs are induced in response to DNA damage[113] and thus could be involved in the development of resistance to therapy.

NATs are another large group of lncRNAs; they are RNA transcripts encoded in the genome that have sequence complementarity to protein-coding RNA transcripts and regulate the transcription of protein-coding genes[21,105]. It has been estimated that approximately one-third of protein-coding genes are regulated by NATs[21]. Interestingly, studies have shown that the NAT regulatory mechanism is more likely to be a consequence of epigenetic modulation (such as DNA methylation induced by the interaction of NATs with DNA methyltransferases and the modification of chromatin structure by the recruitment of histone-modifying enzymes to the genomic locus) rather than the previously presumed direct degradation of the sense transcript through an siRNA-like degradation mechanism for complementary sequences[21,105,114].

The expression levels of T-UCRs are often altered in leukaemias and in solid tumours[106]. This has significant implications for miRNA therapy, as some of the therapeutic effects mediated by miRNAs could be attributed to the targeting of their downstream lncRNAs (for example, miR-155 was shown to directly target T-UCR 160)[106]. Recently, colon cancer-associated transcript 2 (*CCAT2*), a T-UCR that is transcribed from the highly conserved 8q24 cancer risk locus and is upregulated in microsatellite-stable colorectal cancers, was shown to promote oncogenic activity and induce chromosomal instability in colorectal cancers[115]. In addition, *CCAT2* regulates the expression of *MYC*, which is located downstream of the *CCAT2* genomic locus and is involved in the WNT signalling network[115]. These findings suggest that targeting lncRNAs such as *CCAT2* is likely to broadly affect cancer-associated pathways.

According to a newly proposed competing endogenous RNAs (ceRNAs) hypothesis (BOX 2), different types of RNA transcripts can communicate with each other using miRNA response elements, which have been symbolically referred to as letters of a new language[116]. For example, T-UCRs can be regulated by miRNAs via a direct interaction[106]. The tumour suppressor gene *PTEN* and the *PTEN* pseudogene 1 (*PTENP1*), which is a lncRNA sharing a high degree of sequence homology with *PTEN*, are targeted by and can thus compete for the same set of miRNAs (namely, the miR-17, miR-21, miR-214, miR-9 and miR-26 families)[117]. Accordingly, changes in PTENP1 expression indirectly affect PTEN levels by sequestering PTEN-targeted miRNAs. If PTENP1 expression levels decrease, more miRNAs will be available to target PTEN and ultimately downregulate levels of PTEN expression. As a recent addition to the ceRNA mechanism, circRNAs — naturally occurring

RNAs with a circular structure — have attracted much attention because they bind and sequester miRNAs, and thus de-repress the mRNA genes that are normally regulated by the sequestered miRNAs[103].

lncRNA therapeutics. Several features of lncRNAs render these interesting therapeutic targets. First, levels of lncRNA expression are usually lower than those of protein-coding genes, and this may be due to the expression of lncRNAs only in a selected subpopulation of cells[15]. The exclusive expression pattern of lncRNAs in certain types of tissues or cells provides a unique opportunity for specific regulation by lncRNA-targeting therapeutics (TABLE 2). Second, chromatin modification represents one of the main mechanisms of action of lncRNAs, thus a rationale for targeting the interaction of lncRNAs with epigenetic factors such as PRC2 can be envisioned. Third, as many lncRNAs are located in the nucleus[15] and regulate neighbouring gene expression in *cis*, gene-locus-specific regulation can be achieved by lncRNA manipulation. Various lncRNA therapeutics are being investigated, and several companies are also actively developing lncRNA-targeting therapeutics for the treatment of human diseases[21].

One of the strategies (FIG. 3; TABLE 2) to regulate lncRNA function involves applying specifically designed siRNAs against lncRNAs. Although many lncRNAs are predominantly located in the nucleus, and may thus be less accessible than mRNAs to siRNA targeting, several studies have demonstrated the successful knockdown of lncRNAs by siRNAs irrespective of their subcellular localization. For instance, an *in vitro* study showed that siRNA pools can reduce the expression of several lincRNAs to lower than half of their original levels in human cells[111]. In another study, siRNAs designed against *PANDA* (promoter of CDKN1A antisense DNA damage activated RNA), which is a lncRNA that is involved in the DNA damage response, substantially reduced *PANDA* expression and consequently sensitized human fibroblasts to doxorubicin-induced apoptosis[118]. A more recent publication showed that nuclear RNAs are susceptible to knockdown by siRNAs in myotonic dystrophy both *in vitro* and *in vivo*[119].

These studies demonstrate the feasibility of conventional siRNA treatment for the negative regulation of lncRNAs with oncogenic functions. However, to achieve a general high *in vivo* efficacy, chemical modifications that improve stability, binding affinity, interference capacity and cellular uptake are necessary. In cases where an extensive secondary structure or the nucleotide sequence of the lncRNA is unfavourable for an optimal siRNA design, other strategies can be developed to directly target lncRNAs. These include antisense oligonucleotides as well as ribozymes or deoxyribozymes, which utilize different interaction mechanisms with the target lncRNAs (FIG. 3; TABLE 2). Advantages of antisense oligonucleotides over siRNAs include their independence with regard to the RISC machinery, higher specificity and fewer off-target effects[120]. Recent studies have shown antisense oligonucleotides that inhibited MALAT1 function and blocked the metastasis of lung cancer cells in a mouse model[121]. Ribozymes or deozyribozymes (such as hammerhead ribozyme), which bind to a complementary target sequence and catalyse the cleavage of the flanked RNA region, may also be utilized for the targeting of lncRNAs that are are unfavourable for optimal siRNA design because of their short length and extensive secondary structures.[120].

Another approach for targeting lncRNAs involves using synthetic RNA molecules that form hairpin structures that mimic lncRNAs. For example, *GAS5* (growth arrest-specific 5) is a lncRNA that can bind to the glucocorticoid receptor (GCR; also known as NR3C1) and inhibit the interaction of this transcription factor with DNA promoters, functioning as a decoy to block the transcription of target genes[122]. A mutant GAS5 mimic lacking the GCR-binding site failed to inhibit GCR-induced transcription[122]. Based on this observation, the use of synthetic lncRNA mimics to restore the function of endogenous lncRNAs can be envisioned. Conversely, synthetic mutated lncRNAs may be used to competitively block the function of overexpressed cancer-related lncRNAs. However, the length of the lncRNAs may be an obstacle for such applications.

The targeting of NATs represents a unique opportunity for the therapeutic regulation of specific genes[105]. The inhibition of NATs by single-stranded oligonucleotides, termed antagoNATs, has been shown to disrupt the interaction of a NAT with its complementary mRNA and thus increase the expression of the protein-coding gene[123]. To improve stability and cellular uptake, the chemical modifications used for oligonucleotide-based miRNA therapeutics have also been applied to antagoNATs. In a recent study, a 14-nucleotide antagoNAT with a mixture of 2′-*O*-methyl RNA and LNA modifications targeting the brain-derived neurotrophic factor antisense transcript (*BDNF*-AS) efficiently inhibited its function and thus increased transcription of the sense *BDNF* mRNA both *in vitro* and *in vivo*[124]. One advantage of antagoNATs is their ability to increase protein expression, and this upregulation of protein output is difficult to achieve with other conventional drug designs.

Challenges and outlook

The development of miRNA- and lncRNA-targeted strategies is challenged by several obstacles. One is the successful delivery of the therapeutic agent to the target tissues. Oligonucleotide-based therapeutics must overcome obstacles such as their degradation by nucleases, renal clearance, failure to cross the capillary endothelium, ineffective endocytosis by target cells or ineffective endosomal release[125,126]. An additional challenge is the release of RNA-based therapeutics from the blood to the target tissue through the capillary endothelium if these therapeutics form complexes that are larger than 5 nm in diameter[127].

Also, although local delivery into the eye or skin has been shown to improve bioavailability in targeted sites, systemically delivered miRNA formulations and RNA-based miRNA-targeting agents might be negatively influenced by the host immune system, as macrophages and

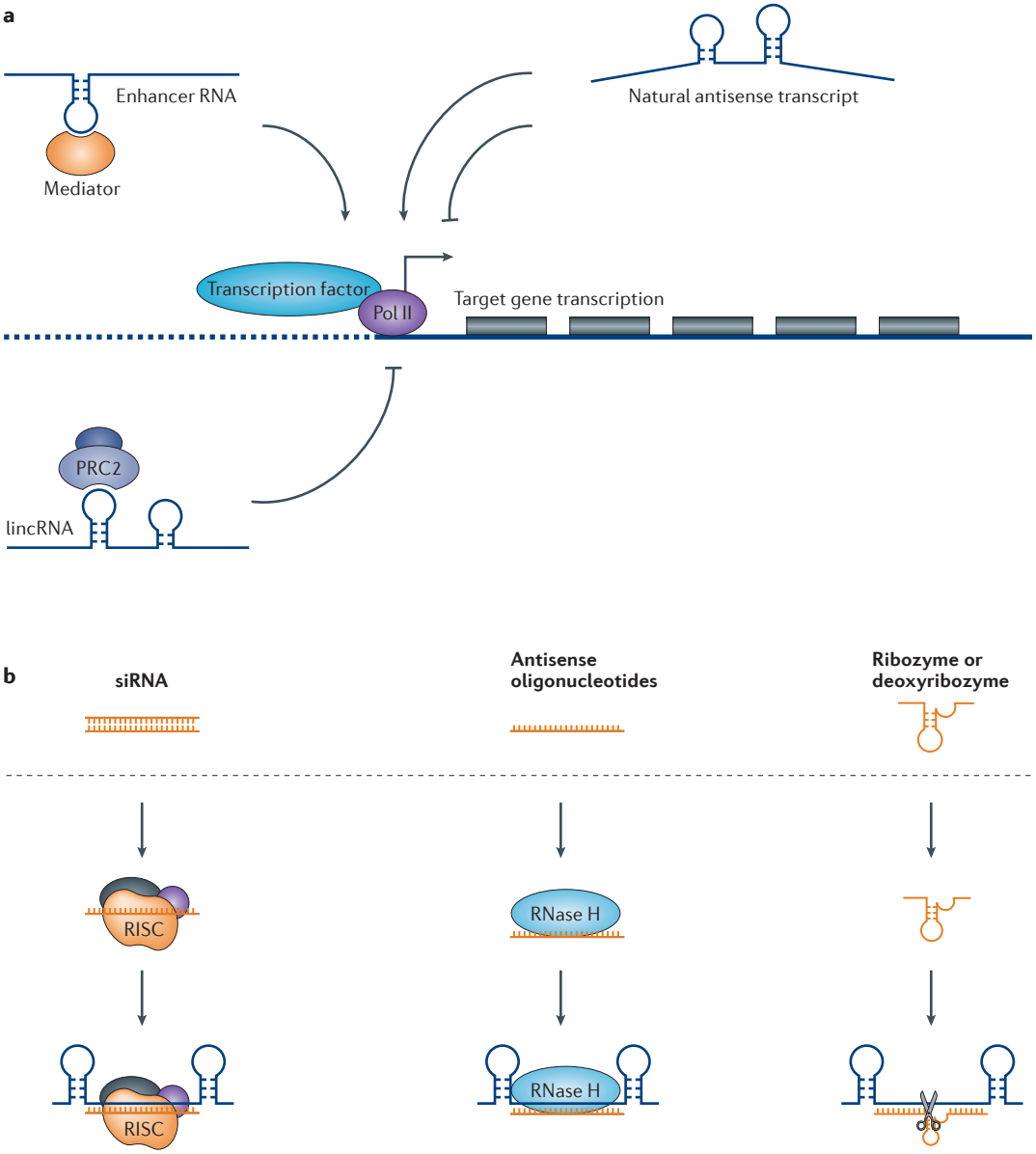

Figure 3 | **Mechanisms of action of lncRNAs and the use of therapeutic agents to regulate their function.** **a** | The mechanisms of action of long non-coding RNAs (lncRNAs) are more diversified than those of microRNAs (miRNAs), and several representative examples are shown. About 20% of long intergenic ncRNAs (lincRNAs) are bound to Polycomb repressive complex 2 (PRC2) and inhibit transcriptional activity by *trans* regulation. Enhancer RNAs (eRNAs) associate with mediator proteins to modify chromatin structure and activate gene transcription in a *cis* manner. Natural antisense transcripts (NATs) are another type of lncRNA and are transcribed from either the same genomic site or a site distant from the gene locus where the sense transcript counterpart is produced. NATs repress — and in some cases can also activate — transcription of the targeted protein-coding genes via mechanisms such as DNA methylation and chromatin modification at the genomic loci of the targeted genes. **b** | Several methods, including small interfering RNAs (siRNAs), antisense oligonucleotides and ribozymes or deoxyribozymes, can be used to block the function of lncRNAs. The double-stranded siRNA duplex can be stably produced by vectors encoding short hairpin RNA (shRNA) or transiently transfected with synthetic double-stranded short RNA. The antisense strand of the siRNA duplex loads on to the RNA-induced silencing complex (RISC) and degrades the targeted lncRNA. Antisense oligonucleotides are single-stranded, chemically modified DNA-like molecules (13–25 nucleotides in length) that are designed to be complementary to a targeted RNA. Antisense oligonucleotides form a heteroduplex with the RNA, and RNase H recognizes the RNA–DNA heteroduplex and cleaves the RNA strand. siRNAs and antisense oligonucleotides are the main tools in most current studies that target lncRNAs. However, ribozymes or deoxyribozymes (regardless of whether they are naturally occurring or artificially synthesized) are ideal candidates for lncRNA therapeutics owing to their unique features such as their specificity in target recognition and their independent activity (they do not rely on the RISC, which mediates siRNA-induced degradation, or on RNase H, which is essential for the activity of antisense oligonucleotides).

monocytes can remove complexed RNAs from extracellular spaces[128]. For instance, double-stranded RNAs that are ≥21 base pairs in length can lead to a sequence-independent interferon response[129]. Moreover, the limitations for the use of siRNA-based therapeutics equally apply to miRNA-based therapeutic methods; these limitations include carrier toxicity (including haemolysis), thrombogenicity and complement activation induced by nanoparticles, and mutagenesis potential with the viral vectors[126]. Notwithstanding these limitations, in 2012 the gene therapy drug alipogene tiparvovec (Glybera; UniQure), an AAV-packed protein-coding gene for the treatment of lipoprotein lipase deficiency, was approved by the European Union[130,131], which indicates the plausibility of vector-based miRNA replacement or miRNA blocking with miRNA sponges in clinical practice.

Another challenge is the safety evaluation of miRNA-based therapeutics, such as the potential immune response to the delivery system, toxicity caused by the chemical modification or unexpected side effects that are likely to occur, given that each miRNA can affect hundreds of target genes. The findings that exosome miRNAs can activate TLRs and promote the secretion of IL-6 and TNF[27] indicate that miRNA mimics may also induce such activities and thus it is necessary to monitor this reaction when using miRNA mimics as cancer therapeutics. This issue is further complicated by the recently identified novel mechanisms of action of miRNAs, such as the upregulation of protein expression or the regulation of protein-coding genes via complementarity to the coding region or the 5′ UTR of the targeted genes. The recent finding that the deletion of the oncogenic miR-17–miR-92 cluster causes human congenital syndromic developmental defects suggests that it is necessary to consider the non-cancer related effects of miRNAs when designing miRNA-based therapeutics for cancer treatment[132]. This is particularly important when long-term delivery is required.

Owing to the complexity and challenges associated with miRNA research, the only miRNA-targeted therapeutic that has been tested in clinical trials so far is miravirsen. However, miRNA therapeutics are in the developmental pipelines of several pharmaceutical companies. For instance, miRagen Therapeutics is currently focusing on the treatment of cardiovascular and muscle diseases via miRNA inhibition and replacement. Regulus Therapeutics is actively exploring the value of anti-miRs in the treatment of diseases such as fibrosis, HCV infection, atherosclerosis and cancer. MRX34 (developed by Mirna Therapeutics), a liposome-formulated mimic of the tumour suppressor miR-34, produced complete tumour regression in two separate orthotopic mouse models of liver cancer, with no observed immunostimulatory activity or toxicity to normal tissues (see the MRX34 product information page on the Mirna Therapeutics website). These results have prompted a clinical Phase I trial (ClinicalTrials.gov identifier: NCT01829971), which is currently recruiting patients with advanced or metastatic liver cancer.

What are the expectations for the future? We predict that new miRNA-targeting anticancer drugs with improved specificity and efficacy will soon enter the clinical stage of development and finally be used, in combination with chemotherapy and radiotherapy, for the treatment of patients with cancer. The main obstacle associated with the clinical application of miRNA-targeting strategies is determining how to precisely deliver the therapeutic agents into the targeted cells without inducing unwanted responses in cells other than the intended ones. Nanoparticles that are specifically engineered for delivery to specific cells will further help with this goal. miRNA dysregulation has also been associated with diseases beyond cancer. Therefore, it can be expected that clinical trials for diseases such as sepsis, which require prompt treatment but only for a short duration, would be very informative with regard to the therapeutic efficacy of miRNAs. However, the possible development of chronic toxicities will need to be taken into consideration in the development of ncRNA therapeutics for cancer.

The novel mechanisms of miRNA action also offer opportunities for miRNA-targeting strategies. Indeed, the development of the first clinically evaluated miRNA-targeting agent is not based on the canonical mechanism of miRNA action but instead initiated on the unusual finding that miR-122 binds to the 5′ non-coding region of the HCV genome and upregulates HCV RNA expression. Similarly, the miRNAs that upregulate protein translation can be targeted to control the translation of specific oncogenes or tumour suppressor genes. It can also be envisioned that blocking the production, transportation and release of exosome miRNAs may have beneficial effects in controlling cancer development, and this may be achieved by targeting other non-cancerous cell such as inflammatory cells in the cancer microenvironment.

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