ELSEVIER

Cancer therapy via modulation of micro RNA levels: a promising future

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Micro RNAs (miRNAs) are a class of naturally occurring ~22 nt long non-coding small RNA molecules that regulate the expression of a wide range of genes involved in development, growth, proliferation and apoptosis. miRNAs are evolutionarily conserved from plants to animals, and they regulate and fine-tune a diverse array of biological processes. Recently, they have been shown to act as either oncogenes or tumor suppressors in a wide variety of tumors. Here, we review the studies that document the role of miRNAs as key players in human cancer and the potential therapeutic modality of exploiting miRNAs for cancer prognosis and treatment.

Introduction

Achieving a complete cure for all types of cancer remains a challenge today, and finding effective anti-cancer therapeutic modalities is one of the major focuses of research worldwide. Active research ranges from cytokine therapy to cancer-specific nanoparticle delivery that promises effective combat against cancer. One of the recent areas at the forefront of modern biology is the non-coding RNAs, specifically the micro RNAs (miRNAs; see Glossary). miRNAs belong to a large family of non-protein-coding RNA molecules that comprises small interfering RNA (siRNA), miRNA, tasiRNA, natsiRNA, tncRNA, scnRNA, rasiRNA, piwi-interacting small RNA, and so on [1]. The discovery of miRNA occurred in 1993, when Lee *et al.* [2] reported that the *Caenorhabditis elegans* gene lin-4 coded for a small antisense RNA complementary to a developmentally regulated protein-coding gene lin-14. Since then, several miRNA genes have been cloned and shown to be evolutionarily conserved from plants to humans and as negative regulators of gene expression [3]. The finding of miRNAs preceded the actual discovery of the phenomenon of RNA interference in 1998 [4], and the existence and mechanisms of biogenesis of several classes of small RNAs have been worked out in great detail [5]. Today, our understanding of miRNA has expanded many times over, from the biology of miRNA synthesis and dysregulation of miRNA expression in many types of cancer to targeting miRNAs for therapy.

miRNAs as novel regulators of gene expression

miRNAs are ~22 nt long small RNA molecules expressed in both plants and animals; they negatively regulate gene expression by binding to perfect or nearly perfect complementary sequences present at the 3′ UTR of target genes. Two major mechanisms have been ascribed to miRNA-mediated gene repression, both of which are post-transcriptional. The first and the predominant mechanism is one in which miRNAs block the translation of gene transcripts by binding to the 3′ UTR, resulting in the accumulation of stalled polyribosomes [6]. The major hallmark of this phenomenon is that only the protein level of the target gene is altered, not the transcript. In the second pathway, binding of miRNAs to the target sequence leads to the degradation of the transcript [7], in a process executed by the machinery employed by the siRNAs. Apart from these two common modes of action, miRNAs are also reported to be involved in transcriptional gene silencing [8].

Most miRNAs are transcribed by RNA polymerase II as capped, polyadenylated large RNA precursors [9] called primary miRNAs, which are processed in the nucleus by an RNase III enzyme called Drosha [10] aided by its co-factor protein (Pasha, or DGCR8). The product of Drosha action is 70 nt long RNA molecules named precursor miRNAs (pre-miRNAs), which form imperfect stem–loop structures (Fig. 1). These pre-miRNAs are transported into the

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GLOSSARY

Antisense-mediated inhibition. Use of antisense oligonucleotides to quench small RNAs through complementary binding. They are usually modified at the ends to enhance stability.
AntagomiRs Antisense oligonucleotides complementary to miRNAs that are upregulated in cancer tissues. AntagomiRs are usually modified at the ends to enhance stability and thermodynamics of binding.
Asymmetric siRNA (asiRNA) Second-generation RNAi effector designed to favor antisense strand selection by ways such as introduction of unilateral overhangs in the ends of the antisense strand. Shown to reduce off-target effects.
Micro RNA (miRNA) miRNAs are small 22 nt long RNA molecules that are processed from long double-stranded hairpin duplex in a process similar to siRNA, but they act through translational repression via imperfect biding to the 3′ UTR of target mRNA.
miRNA replacement therapy. Administration of sense microRNA oligonucleotides or to supplement the lowered levels of specific miRNA in cancer tissues.
OncomiRs miRNAs whose expression levels are specifically upregulated or downregulated in cancer tissues compared with normal counterparts.
Piwi-interacting small RNA Piwi-interacting small RNAs are similar to rasiRNA in terms of biogenesis but are not produced from repeat associated regions in the genome. They are implicated in transposon control in germlines, although the precise mechanism is not well characterized.
RNA:DNA chimeric duplex Second-generation RNAi effector wherein parts of the dsRNA are replaced with cognate deoxynucleotide and are shown to eliminate off-target effects.
Small interfering RNA (siRNA) siRNAs are small 18–22 nt long RNA molecules that are processed from long double-stranded hairpin duplex. They act primarily through targeting mRNA cleavage through perfect complementarity in the coding region.
Small internally segmented RNAi Second-generation RNAi effector wherein the sense strand is internally segmented and held together by locked nucleic acid. Shown to reduce off-target effects mediated by sense strand.
Tandem hairpinRNA Delivery of multiple shRNA effectors can be achieved using tandem hairpinRNA, wherein two or more shRNAs are placed in tandem. Suggested to have reduced exportin-5-mediated toxicity.

cytoplasm via the exportin-5 complex [11] and, subsequently, processed by another RNase III enzyme, Dicer, to produce 22 nt long mature miRNAs [12]. The double-stranded mature miRNAs are then bound by a protein complex including Ago and other dsRNA-binding proteins, such as TRBP, to form the miRNA-associated RNA-induced silencing complex (miRISC) [13]. On the basis of relative thermodynamic stability of the miRNA strands, one strand is selected to be the guiding strand or the miRNA and the other strand is degraded [14]. The guiding strand is selectively retained on the RISC complex, which directs the binding of miRNA to its complementary sequence at the 3′ UTR of the target transcript. The pathway of miRNA biogenesis and the mechanism of action of miRNAs largely overlap with those of siRNA [15]. There are some major differences between miRNA and siRNA pathways, however: (i) miRNA binds to non-perfect complementary sequences in the target transcript, whereas siRNAs require perfect sequence complementarity [5]; (ii) miRNA-binding sites mostly occur at the 3′ UTR of the transcript, whereas siRNA-binding sites occur at the transcript-coding region [16] (it is important, though, to mention that recent studies suggest that miRNAs could bind to target sites in the coding region of target mRNA as well [17,18]); and (iii) siRNAs are highly specific to individual gene targets, whereas a single miRNA can bind to multiple transcripts and, in addition, each gene target can have multiple miRNA-binding sites [19]. Not only is the mechanism of action of miRNA fascinating, but also control of their expression seems to be complex; it is mediated by both transcriptional and post-transcriptional mechanisms, including (but not limited to) regulation of miRNA processing, nuclear export and miRNA editing [20].

OncomiRs

With the discovery of lin-4 and let-7, thousands of miRNAs have been cloned and demonstrated to regulate several biological processes, including development [16,21], growth [22] and apoptosis [23]. The pattern of miRNA expression varies among different tissue lineages [24] and within each cell type, and the expression varies as the cell responds to different environmental cues. Because they influence a wide variety of biological processes, miRNAs have emerged as biomarkers of distinct biological states such as cancerous vs. normal tissues [25,26], differentiated vs. stem cells [17,27], and so on. Literature has documented several miRNAs that are either upregulated or downregulated in cancer cells, which are collectively termed 'oncomiRs' [28]. In recent years, however, consensus has emerged that there is a global downregulation of miRNAs in cancer tissues compared to their normal counterparts, suggesting that most of the miRNAs might act as tumor suppressors, the prominent ones being let-7 family miRNAs [29] and the p53-regulated miR-34 family [30]. Recently, several miRNAs have also been shown to be amplified or upregulated in cancer cells and function as oncogenes [31]. Notable examples include the miR17-92 cluster, which is amplified in lymphomas and lung cancer [32,33], and miR-21 upregulation in many solid tumors, including pancreas and breast cancers [34]. The list of oncomiRs is growing longer (Fig. 2), with many of them being validated *in vitro* and *in vivo* in mice models; this, together with human patient studies, should enable us to identify potential miRNA targets for cancer therapy.

miRNA in cancer prognosis

miRNA profiling of cancer and normal tissues has emerged as a powerful means to diagnose, classify tumor type and tumor grade, and offer valuable prognostic details for various cancers [25,26]. miRNA profiling can be achieved by various techniques, including microarray [32], quantitative PCR [35], and bead-based flow cytometry assays [25]. Non-invasive methods including detection of miRNAs in cancer patient sera or urine are emerging as novel methods of cancer diagnosis and prognosis. For example, serum levels of miR-155, miR-210 and miR-21 hint at a reliable diagnosis of diffuse B-cell lymphoma and relapse-free survival, as reported by Lawrie *et al.* [36]. Similarly, urinary levels of miR-126 and miR-182 have been found to be diagnostic markers for urinary bladder cancer [37]. Large-scale patient sample studies attest to the

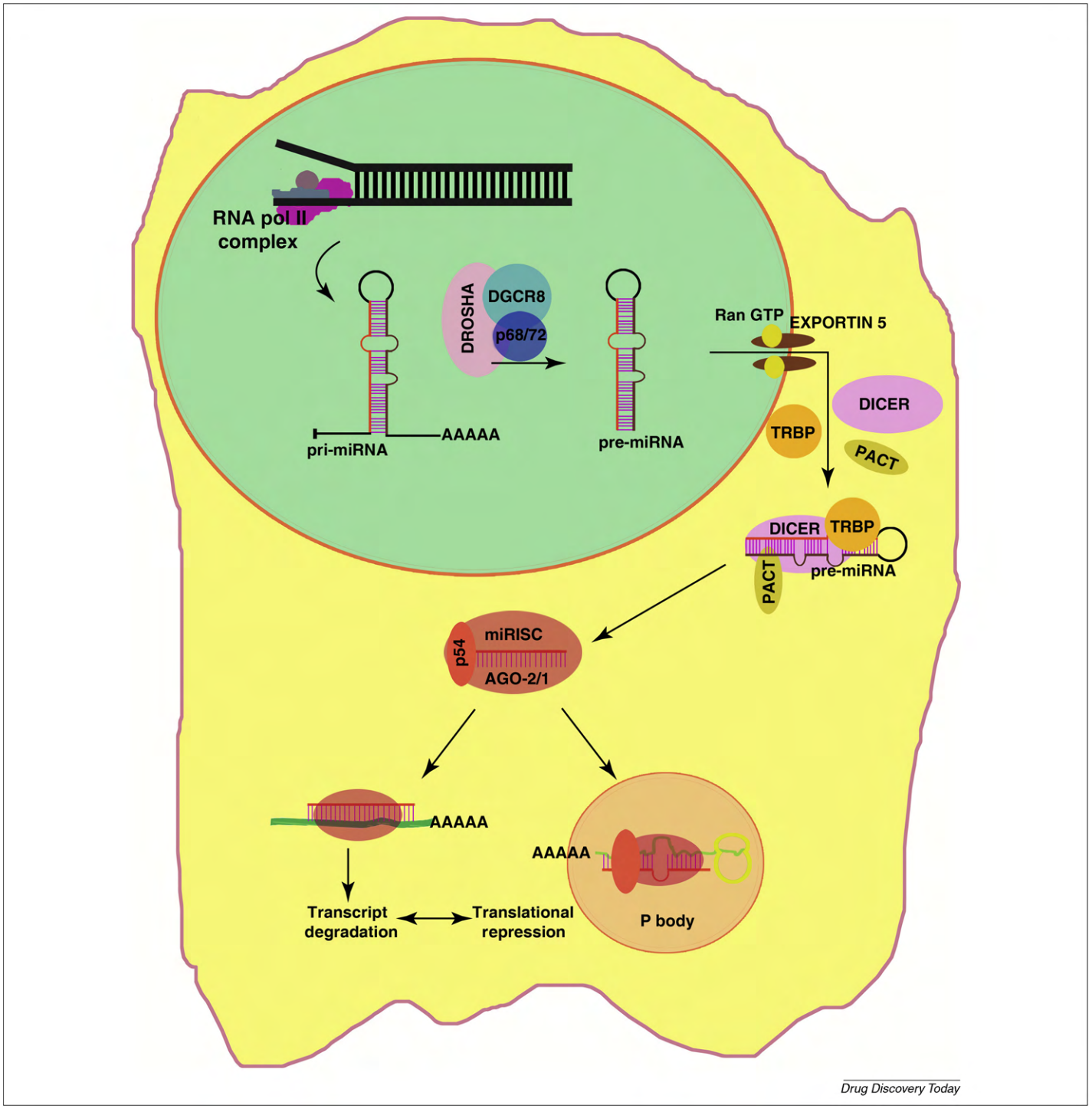

FIGURE 1

miRNA biogenesis and action. miRNAs are transcribed by polymerase II as long stem–loop transcripts – namely, primary miRNAs (pri-miRNAs) – and are processed by RNase III enzyme DROSHA into precursor miRNAs (pre-miRNAs) in the nucleus, exported into cytoplasm by EXPORTIN-5. In the cytoplasm, pre-miRNAs are further processed to 21–23 nt long mature miRNAs by DICER. The guide strand of miRNA duplex is then loaded onto the RNA-induced silencing complex (RISC) containing Argonaute proteins (AGO), forming miRISC. The sequence homology of guide miRNA to the target facilitates recruitment of specific miRISC to the target. This is followed by either target degradation or translational repression resulting in the silencing of the target gene expression.

usefulness of miRNA profiling in cancer prognosis and responsiveness to therapy. In a study of non-small-cell lung cancer, the expression level of miR34a, a direct p53 target, emerged as a reliable prognostic marker for the relapse of the disease [38]. In this study, both cancer and normal tissues were analyzed from 70 surgically resected patients with no post-surgical treatment until relapse. Both lower expression and promoter methylation of miR-34a were found to be associated with non-small-cell lung cancer relapse. A cohort study involving 455 liver cancer patients found that overall, those whose tumors had higher levels of miR-26 lived four years longer than those with lower levels of miR-26 [39]. In addition, in this study, it was found that those having low miR-26

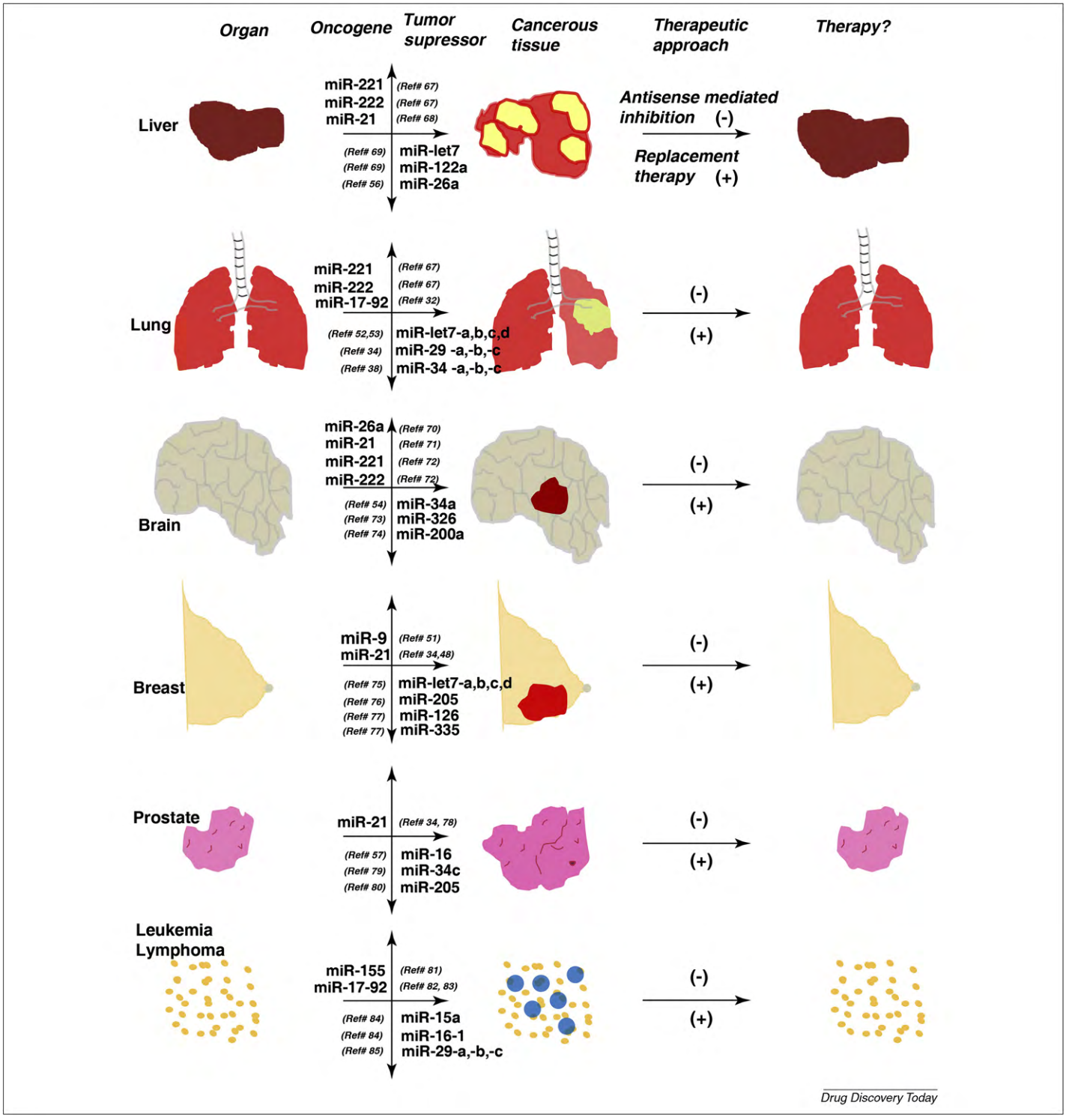

FIGURE 2

Potential miRNA targets for cancer therapy. The figure summarizes some of the miRNAs that are reported to be upregulated (upward arrow) or downregulated (downward arrow) in various cancers (organ) and have been shown to alter cell growth, apoptosis or invasiveness of the cancer cells. The literature references are provided alongside each miRNA. miRNAs that serve as oncogenes could be targeted by antisense-mediated inhibition of the miRNA (−) using antagomiRs or complementary sequestering sequences, and those that function as tumor suppressors could be targeted by miRNA replacement therapy (+) via overexpression of mature or primary microRNAs. By employing tested and proven nucleic-acid-based therapeutic agents (anti-miRs and miRNA mimics), targeted regulation of miRNA levels in cancer tissues could be achieved, resulting in possible cancer therapy.

levels responded to interferon alpha therapy and lived 7.5 years longer than those who did not receive the therapy. Furthermore, women have higher levels of miR-26 in liver tissues compared to men and are less susceptible to liver cancer.

Not only the levels of miRNAs but also the levels of small RNA processing enzymes Dicer and Drosha reflect the oncogenic status of a cell. Merritt *et al.* [40] reported that the mRNA levels of Dicer and Drosha were reduced in 50–60% of ovarian cancers, which

directly correlates with their protein level. Low Dicer level was found to be associated with high-grade tumor, and increased levels of Dicer and Drosha are associated with increased median survival. Similarly, Karube *et al.* [41] reported that reduced level of Dicer expression in lung cancer has a profound prognostic value in determining the survival of surgically treated patients.

Single-nucleotide polymorphism (SNP) analysis of miRNA-binding sites in target genes has revealed prognostic details in various cancer types. He *et al.* [42] reported that in papillary thyroid cancer cells, miR-221 target KIT has harbored SNPs in the 3′ UTR corresponding to the miR-binding site. Another study [43] found a prevalence of SNPs in KRAS, a well known oncogene and a target of let-7, in ~20% of the non-small-cell lung cancer patients. A recent report shows the association of genetic variation in the miRNA sequence with breast cancer risk. The SNP rs895819 is located in the terminal loop of pre-miRNA 27a, an oncogene, and predicted to block the maturation of miR-27a and, therefore, affect cancer risk [44]. SNPs in pre-miRNAs of hsa-mir-146a, hsa-mir-149, hsa-mir-196a2 and hsa-mir-499 affect their processing and are associated with breast cancer risk [45]. Thus, miRNA expression levels and allelic information about miRNAs and their targets are emerging as useful tools to predict cancer risk, cancer grade, therapeutic responsiveness and overall prognosis of the disease. Validated miRNAs and their targets could serve as potential biomarkers in cancer screening measures.

miRNAs as therapeutic targets

With the advances in understanding of the biological network of several miRNAs that plays a crucial part in a wide range of tumors, there has been steep progress in targeting these miRNAs to effectively stall tumor progression *in vivo* in mice. These studies promise an immediate translation into a powerful modality of treating human cancer when coupled with human patient studies and research to enhance biosafety, bioavailability and pharmacokinetics of miRNA-or -pathway targeting molecules. The miRNA-targeting approach comprises miRNA inhibition therapy and miRNA replacement therapy, which are discussed in detail below. Cancer tissues are marked by specific upregulation or downregulation of certain miRNAs compared to normal tissues. Use of anti-miRNA oligonucleotides (antagomiRs) against oncogenic miRNAs that are upregulated in cancers and miRNA replacement therapy for tumor suppressor miRNAs that are downregulated specifically in tumors, therefore, are to be well tolerated by normal tissues without any adverse effect (Fig. 2). As more details emerge from studies of individual miRNA and its role in oncogenesis, progression or suppression, as well as its interacting network in specific cancer type, miRNA-based cancer therapy is likely to be a far-reaching modality for cancer therapy in the near future.

Antisense-mediated inhibition of oncogenic miRNAs

A lot of research has been done on exploiting siRNA-based therapeutic modalities in the past decade, and antisense oligonucleotide (ASO)-based inhibition of oncogenic miRNAs has been a straightforward approach to relieve target repression from miRNAs. ASOs could potentially be designed to block Watson–Crick base pairing at any of the several important intermediary steps during the biogenesis and action of miRNA, including blocking miRNA processing, miRNA loading onto RISC and/or miRNA pairing with target mRNA. Strand replacing oligonucleotides that are complementary to the guiding strand of the miRNA duplex are the most obvious choice of ASO-mediated miRNA inhibition. Several *in vitro* and *in vivo* mice studies have used chemically modified ASOs against oncogenic miRNAs to induce cancer cell death and inhibit tumor proliferation, migration and invasion. For example, inhibition of miR17-5 and miR-20a belonging to the miR-17-92 miRNA cluster (which are overexpressed in B-cell lymphomas and lung cancer) using ASOs resulted in the induction of apoptosis in lung cancer cells [46]. In a similar study, Sylvestre *et al.* [47] found that the inhibition of the miR17-92 cluster using ASOs conferred apoptosis of prostate cancer cells by relieving the translational repression of E2F family proteins. Likewise, ASO against miR-21 (which was found to be differentially overexpressed in breast cancer cells) resulted in the inhibition of breast cancer cell growth both *in vitro* and *in vivo* in a xenograft model [48]. The inhibition of cell proliferation was accompanied by increased cellular apoptosis. Recently, Segura *et al.* [49] reported that antisense-mediated repression of miR-182 resulted in the inhibition of melanoma invasion and also induced cell death.

Besides antisense-mediated inhibition of oncogenic miRNAs, another technique has emerged as an effective way to quench the higher levels of these miRNAs: namely, miRNA sponges [50]. In this approach, specific miRNA target sequences are cloned in multiple copies in tandem at the 3′ UTR of a transcript that is driven by a strong promoter. Essentially, upon transfection into a tumor cell, such a construct should act as a sponge for the respective miRNA that is targeted and relieve its natural target from repression. Recently, a miRNA sponge against miR-9, an oncomiR found to be upregulated in breast cancer cells and implicated in cancer metastasis, effectively abrogated the invasiveness of the tumor cells [51].

miRNA replacement therapy

In this approach, miRNAs that act as tumor suppressors and are generally downregulated in tumors are replaced by exogenous administration of synthetic miRNAs (i.e. pre-miRNAs or mature-miRNAs). Let-7 is a proven tumor suppressor miRNA that is downregulated in several cancer types – including ovarian, prostate, lung, breast, pancreas, melanoma, mesothelioma and lymphoma – and apparently also in cancer stem cells. In a recent study, exogenous delivery of let-7 to established tumors in a mouse model of non-small-cell lung cancer led to the reduction of tumor burden [52]. In this study, let-7 oligonucleotide was delivered intra-tumorally, resulting in the regression of non-small-cell lung carcinoma, suggesting that miRNA replacement therapy could be a potential way of treating these tumors in humans in the future. In addition, anti-let-7 treatment exacerbated the tumor growth corroborating the phenotype of let-7 replacement therapy. This study and reports from other groups [53] suggest that let-7 acts as a tumor suppressor through direct repression of KRAS, HMGA2 and c-myc oncogenes.

miR-34, a direct target of p53, has been implicated as a tumor suppressor often downregulated in a variety of cancers, including gastric cancer, pancreatic cancer, human glioma and medulloblastoma [54] and targets Bcl2, Notch and HMGA2. In human gastric

cancer cells, introduction of miR-34 mimic (oligonucleotide) by transfection or by lentiviral-mediated delivery resulted in cell-cycle arrest at G1 and impaired cell growth, sensitized the cells to cell-death-inducing agents and reduced tumorsphere formation [55]. In an interesting study reported by Kota *et al.* [56], miR-26a underexpressed in hepatocellular cancers when delivered by adeno-associated viral delivery system potently suppressed tumor progression. In this cancer model, miR-26a does not target the tumor-initiating oncogene but directly targets cyclins D2 and E2 and induces G1 arrest in human liver cancer cells. In addition, miR-26a administration induced tumor-specific apoptotic pathway, as normal cells express high levels of miR-26a.

In yet another study, systemic delivery of miR-16, which is downregulated in prostate cancer cells, was shown to reduce cancer cell proliferation [57]. miR-16 complexed with atelocollagen, when administered systemically via tail vein, prevented growth of prostate cancer cells in a bone metastasis model in mice, indicating the therapeutic application of treating advanced prostate cancer patients with miR-16 replacement therapy.

These findings in mice prove that systemic delivery of tumor suppressor miRNAs could be a highly tumor-specific therapeutic modality with no toxicity to normal cells. Further research on enhancing miRNA accessibility to tumor and half-life would dramatically improve the arsenal in the combat against cancer.

The helper role(s) of miRNA in cancer therapy

Not only do miRNAs act as direct effectors in anti-cancer therapy but also the biological specificity of their action and tissue specificity of endogenous expression could potentially be exploited in facilitating specific delivery of lethal anti-cancer drugs and oncolytic viruses to cancer cells, avoiding the common cytotoxic effects generally observed in liver and kidney tissues.

A clever study [58] reported the use of miR-122a target sequence in an adenoviral ganciclovir delivery vector, with a lowered transgene expression of up to 1500-fold in hepatic tissues compared to conventional adenoviral vectors. Such suicide gene therapy could be devised with greater target specificity by using the knowledge of miRNA expression profiles and their targets coupled with ingenuity to address specific toxicity issues. Similarly, alteration of virus or vector tropism by inserting specific miRNA target sequence in the 3′ UTR of replication-incompetent adenoviruses and lentiviruses is a promising strategy in targeting cancer cells specifically, which could be extended to potent oncolytic, replication-competent viruses. In an elegant study [59], the tropism of a deadly picornavirus was limited to cancer cells, avoiding the common side-effect of lethal myositis. This was achieved by attenuating the replication of the oncolytic virus in muscle cells by inserting a tandem repeat of miR-303T, miR-206T at the 3′ UTR of the viral genome. The modified virus retained oncolytic activity, while the mice were protected from myositis.

In another study, oncolytic herpes simplex virus (a proven oncolytic virus being tried in many phase I clinical trials to treat cancer) was engineered to harbor target sequences for miR-143 and miR-145, which are normally downregulated in prostate cancer cells [60]. The study showed an ~80% reduction of tumor burden in a xenograft mice model of human prostate cancer, and the miRNA-regulated viral amplicons were detected specifically in the tumor cells and not in the normal tissues. Thus, incorporating miRNA-targeting sequences in therapeutic vectors and viruses has emerged as a great tool to define specificity and reduce toxicity.

Stability and delivery modes of miRNA-targeting molecules

As with any drug delivery system, stability, pharmacokinetics and the delivery modes determine the success of miRNA mimics or miRNA ASO-mediated cancer therapy. Over the past decade, research conducted in the field of siRNA-mediated therapeutic strategy has resulted in an in-depth knowledge of the ASO-based *in vivo* therapeutic issues such as toxicity, stability, specificity, and so on. Some of the potential ways to enhance miRNA stability include chemical modifications (such as locked nucleic acid, 2′-O-methyl, 2′-O-methoxyethyl, morpholinos and phosphorothioate modifications) that have tremendously improved the affinity, stability and serum half-life of the ASO while preserving their specificity of binding. An elegant study reported ASOs termed 'antagomiRs', which are cholesterol conjugated and 2′-O-methyl modified [61] and exhibited improved stability and thermodynamics. In this study, intravenous administration of antagomiRs against miR-122, miR-16, miR-192 and miR-194 resulted in specific, long-lasting reduction in the corresponding miRNA levels in several tissue types including liver, kidney, lung, heart, bone marrow and muscle. The antagomiR strategy was recently tested in a non-human primate model, which demonstrated that systemic delivery of unconjugated locked nucleic acid-modified ASO against miR-122 effectively antagonized miR-122 levels in liver [62], promising therapeutic efficacy in humans.

Several different options are available for the delivery of antagomiRs and miRNA mimics, including oral, intravenous, subcutaneous, nasal, intraocular and gut-directed delivery. Broadly, these could be categorized into local and systemic [63]; delivery agents could vary from liposomes, electrotransfer and attenuated viruses to nanoparticles [64,65]. Recent research in small-RNA-based therapeutics has yielded several different second-generation RNAi effectors that improve their stability, effectiveness and specificity. These include asymmetric siRNA, small internally segmented RNAi, RNA:DNA chimeric duplex, trans-kingdom RNAi, pre-miRNA mimics, tandem hairpin RNA [66], and so on, which could help further the efficacy of miRNA-based cancer therapeutics.

Future outlook

Currently, small RNA research is at its peak, with much public and private research focused not only on understanding the biology but also on advancing the therapeutic use of targeting miRNAs in treating various human illnesses, from treating viral infections and correcting cellular metabolism to curing cancer. Thus far, research conducted in mice, regulating cancer-specific miRNA levels and a lone primate study employing antisense miRNA strategy are highly encouraging towards translating into human cancer therapy. Extensive research carried out in the past decade exploring siRNA-based therapeutic strategies, some having already passed through several stages of clinical trials, could be valuable in addressing human-specific issues such as toxicity, availability, circulating half-life and so on in miRNA-based cancer therapeutic

approaches. The day when miRNA-based drugs will be used for the clinical treatment of cancer, which might offer a safe and cancer-tissue-specific treatment modality and aid the detection and prognosis of the disease, does not seem far off.

References

1 Farazi, T.A. *et al.* (2008) The growing catalog of small RNAs and their association with distinct Argonaute/Piwi family members. *Development* 135, 1201–1214
2 Lee, R.C. *et al.* (1993) The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 75, 843–854
3 Pasquinelli, A.E. *et al.* (2000) Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. *Nature* 408, 86–89
4 Fire, A. *et al.* (1998) Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 391, 806–811
5 Bartel, D.P. (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116, 281–297
6 Olsen, P.H. and Ambros, V. (1999) The lin-4 regulatory RNA controls developmental timing in *Caenorhabditis elegans* by blocking LIN-14 protein synthesis after the initiation of translation. *Dev. Biol.* 216, 671–680
7 Lim, L.P. *et al.* (2005) Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature* 433, 769–773
8 Kim, D.H. *et al.* (2008) MicroRNA-directed transcriptional gene silencing in mammalian cells. *Proc. Natl. Acad. Sci. U.S.A.* 105, 16230–16235
9 Lee, Y. *et al.* (2004) MicroRNA genes are transcribed by RNA polymerase II. *EMBO J.* 23, 4051–4060
10 Lee, Y. *et al.* (2003) The nuclear RNase III Drosha initiates microRNA processing. *Nature* 425, 415–419
11 Bohnsack, M.T. *et al.* (2004) Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. *RNA* 10, 185–191
12 Bernstein, E. *et al.* (2001) Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* 409, 363–366
13 Chendrimada, T.P. *et al.* (2005) TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. *Nature* 436, 740–744
14 Tomari, Y. *et al.* (2004) A protein sensor for siRNA asymmetry. *Science* 306, 1377–1380
15 Carthew, R.W. and Sontheimer, E.J. (2009) Origins and mechanisms of miRNAs and siRNAs. *Cell* 136, 642–655
16 Wightman, B. *et al.* (1991) Negative regulatory sequences in the lin-14 3′-untranslated region are necessary to generate a temporal switch during *Caenorhabditis elegans* development. *Genes Dev.* 5, 1813–1824
17 Tay, Y. *et al.* (2008) MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. *Nature* 455, 1124–1128
18 Forman, J.J. *et al.* (2008) A search for conserved sequences in coding regions reveals that the let-7 microRNA targets Dicer within its coding sequence. *Proc. Natl. Acad. Sci. U.S.A.* 105, 14879–14884
19 Lewis, B.P. *et al.* (2003) Prediction of mammalian microRNA targets. *Cell* 115, 787–798
20 Kai, Z.S. and Pasquinelli, A.E. (2010) MicroRNA assassins: factors that regulate the disappearance of miRNAs. *Nat. Struct. Mol. Biol.* 17, 5–10
21 Hornstein, E. *et al.* (2005) The microRNA miR-196 acts upstream of Hoxb8 and Shh in limb development. *Nature* 438, 671–674
22 Brennecke, J. *et al.* (2003) bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in Drosophila. *Cell* 113, 25–36
23 Ambros, V. (2003) MicroRNA pathways in flies and worms: growth, death, fat, stress, and timing. *Cell* 113, 673–676
24 Landgraf, P. *et al.* (2007) A mammalian microRNA expression atlas based on small RNA library sequencing. *Cell* 129, 1401–1414
25 Lu, J. *et al.* (2005) MicroRNA expression profiles classify human cancers. *Nature* 435, 834–838
26 Hernando, E. (2007) microRNAs and cancer: role in tumorigenesis, patient classification and therapy. *Clin. Transl. Oncol.* 9, 155–160
27 Kashyap, V. *et al.* (2009) Regulation of stem cell pluripotency and differentiation involves a mutual regulatory circuit of the NANOG, OCT4, and SOX2 pluripotency transcription factors with polycomb repressive complexes and stem cell microRNAs. *Stem Cells Dev.* 18, 1093–1108
28 Esquela-Kerscher, A. and Slack, F.J. (2006) Oncomirs—microRNAs with a role in cancer. *Nat. Rev. Cancer* 6, 259–269
29 Morris, J.P., IV and McManus, M.T. (2005) Slowing down the Ras lane: miRNAs as tumor suppressors? *Sci. STKE* 297, pe41
30 He, L. *et al.* (2007) A microRNA component of the p53 tumour suppressor network. *Nature* 447, 1130–1134
31 Hammond, S.M. (2006) MicroRNAs as oncogenes. *Curr. Opin. Genet. Dev.* 16, 4–9
32 He, L. *et al.* (2005) A microRNA polycistron as a potential human oncogene. *Nature* 435, 828–833
33 Hayashita, Y. *et al.* (2005) A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. *Cancer Res.* 65, 9628–9632
34 Volinia, S. *et al.* (2006) A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc. Natl. Acad. Sci. U.S.A.* 103, 2257–2261
35 Yu, S.L. *et al.* (2008) MicroRNA signature predicts survival and relapse in lung cancer. *Cancer Cell* 13, 48–57
36 Lawrie, C.H. *et al.* (2008) Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. *Br. J. Haematol.* 141, 672–675
37 Hanke, M. et al.: A robust methodology to study urine microRNA as tumor marker: microRNA-126 and microRNA-182 are related to urinary bladder cancer. *Urol. Oncol.* (in press).
38 Gallardo, E. *et al.* (2009) miR-34a as a prognostic marker of relapse in surgically resected non-small-cell lung cancer. *Carcinogenesis* 30, 1903–1909
39 Ji, J. *et al.* (2009) MicroRNA expression, survival, and response to interferon in liver cancer. *N. Engl. J. Med.* 361, 1437–1447
40 Merritt, W.M. *et al.* (2008) Dicer, Drosha, and outcomes in patients with ovarian cancer. *N. Engl. J. Med.* 359, 2641–2650
41 Karube, Y. *et al.* (2005) Reduced expression of Dicer associated with poor prognosis in lung cancer patients. *Cancer Sci.* 96, 111–115
42 He, H. *et al.* (2005) The role of microRNA genes in papillary thyroid carcinoma. *Proc. Natl. Acad. Sci. U.S.A.* 102, 19075–19080
43 Chin, L.J. *et al.* (2008) A SNP in a let-7 microRNA complementary site in the KRAS 3′ untranslated region increases non-small cell lung cancer risk. *Cancer Res.* 68, 8535–8540
44 Yang, R. *et al.* (2010) A genetic variant in the pre-miR-27a oncogene is associated with a reduced familial breast cancer risk. *Breast Cancer Res. Treat.* 121, 693–702
45 Hu, Z. *et al.* (2009) Common genetic variants in pre-microRNAs were associated with increased risk of breast cancer in Chinese women. *Hum. Mutat.* 30, 79–84
46 Matsubara, H. *et al.* (2007) Apoptosis induction by antisense oligonucleotides against miR-17-5p and miR-20a in lung cancers overexpressing miR-17-92. *Oncogene* 26, 6099–6105
47 Sylvestre, Y. *et al.* (2007) An E2F/miR-20a autoregulatory feedback loop. *J. Biol. Chem.* 282, 2135–2143
48 Si, M.L. *et al.* (2007) miR-21-mediated tumor growth. *Oncogene* 26, 2799–2803
49 Segura, M.F. *et al.* (2009) Aberrant miR-182 expression promotes melanoma metastasis by repressing FOXO3 and microphthalmia-associated transcription factor. *Proc. Natl. Acad. Sci. U.S.A.* 106, 1814–1819
50 Ebert, M.S. *et al.* (2007) MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. *Nat. Methods* 4, 721–726
51 Ma, L. *et al.* (2010) miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis. *Nat. Cell Biol.* 12, 209–211
52 Trang, P. *et al.* (2009) Regression of murine lung tumors by the let-7 microRNA. *Oncogene* 29, 1580–1587
53 Kumar, M.S. *et al.* (2008) Suppression of non-small cell lung tumor development by the let-7 microRNA family. *Proc. Natl. Acad. Sci. U. S. A.* 105, 3903–3908
54 Li, Y. *et al.* (2009) MicroRNA-34a inhibits glioblastoma growth by targeting multiple oncogenes. *Cancer Res.* 69, 7569–7576
55 Ji, Q. *et al.* (2008) Restoration of tumor suppressor miR-34 inhibits human p53-mutant gastric cancer tumor spheres. *BMC Cancer* 8, 266
56 Kota, J. *et al.* (2009) Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. *Cell* 137, 1005–1017
57 Takeshita, F. *et al.* (2010) Systemic delivery of synthetic microRNA-16 inhibits the growth of metastatic prostate tumors via downregulation of multiple cell-cycle genes. *Mol. Ther.* 18, 181–187
58 Suzuki, T. *et al.* (2008) miR-122a-regulated expression of a suicide gene prevents hepatotoxicity without altering antitumor effects in suicide gene therapy. *Mol. Ther.* 16, 1719–1726
59 Kelly, E.J. *et al.* (2008) Engineering microRNA responsiveness to decrease virus pathogenicity. *Nat. Med.* 14, 1278–1283
60 Lee, C.Y. *et al.* (2009) MicroRNA regulation of oncolytic herpes simplex virus-1 for selective killing of prostate cancer cells. *Clin. Cancer Res.* 15, 5126–5135

61 Krutzfeldt, J. *et al.* (2005) Silencing of microRNAs *in vivo* with 'antagomirs'. *Nature* 438, 685–689

62 Elmen, J. *et al.* (2008) LNA-mediated microRNA silencing in non-human primates. *Nature* 452, 896–899

63 Sepp-Lorenzino, L. and Ruddy, M. (2008) Challenges and opportunities for local and systemic delivery of siRNA and antisense oligonucleotides. *Clin. Pharmacol. Ther.* 84, 628–632

64 Li, W. and Szoka, F.C., Jr (2007) Lipid-based nanoparticles for nucleic acid delivery. *Pharm. Res.* 24, 438–449

65 Li, S.D. and Huang, L. (2008) Targeted delivery of siRNA by nonviral vectors: lessons learned from recent advances. *Curr. Opin. Invest. Drugs* 9, 1317–1323

66 Sibley, C.R. *et al.* (2010) Novel RNA-based strategies for therapeutic gene silencing. *Mol. Ther.* 18, 466–476

67 Garofalo, M. *et al.* (2009) miR-221&222 regulate TRAIL resistance and enhance tumorigenicity through PTEN and TIMP3 downregulation. *Cancer Cell* 16, 498–509

68 Meng, F. *et al.* (2007) MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology* 133, 647–658

69 Gramantieri, L. *et al.* (2007) Cyclin G1 is a target of miR-122a, a microRNA frequently down-regulated in human hepatocellular carcinoma. *Cancer Res.* 67, 6092–6099

70 Huse, J.T. *et al.* (2009) The PTEN-regulating microRNA miR-26a is amplified in high-grade glioma and facilitates gliomagenesis *in vivo*. *Genes Dev.* 23, 1327–1337

71 Chan, J.A. *et al.* (2005) MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Res.* 65, 6029–6033

72 Zhang, C. *et al.* (2009) Co-suppression of miR-221/222 cluster suppresses human glioma cell growth by targeting p27kip1 *in vitro* and *in vivo*. *Int. J. Oncol.* 34, 1653–1660

73 Kefas, B. *et al.* (2009) The neuronal microRNA miR-326 acts in a feedback loop with notch and has therapeutic potential against brain tumors. *J. Neurosci.* 29, 15161–15168

74 Saydam, O. *et al.* (2009) Downregulated microRNA-200a in meningiomas promotes tumor growth by reducing E-cadherin and activating the Wnt/beta-catenin signaling pathway. *Mol. Cell. Biol.* 29, 5923–5940

75 Yu, F. *et al.* (2007) let-7 regulates self renewal and tumorigenicity of breast cancer cells. *Cell* 131, 1109–1123

76 Gregory, P.A. *et al.* (2008) The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat. Cell Biol.* 10, 593–601

77 Tavazoie, S.F. *et al.* (2008) Endogenous human microRNAs that suppress breast cancer metastasis. *Nature* 451, 147–152

78 Ribas, J. and Lupold, S.E. (2010) The transcriptional regulation of miR-21, its multiple transcripts, and their implication in prostate cancer. *Cell Cycle* 9, 923–929

79 Hagman, Z. *et al.,* miR-34c is down regulated in prostate cancer and exerts tumor suppressive functions. *Int. J. Cancer.* (in press).

80 Gandellini, P. *et al.* (2009) miR-205 Exerts tumor-suppressive functions in human prostate through down-regulation of protein kinase Cepsilon. *Cancer Res.* 69, 2287–2295

81 Eis, P.S. *et al.* (2005) Accumulation of miR-155 and BIC RNA in human B cell lymphomas. *Proc. Natl. Acad. Sci. U.S.A.* 102, 3627–3632

82 Mavrakis, K.J. *et al.* (2010) Genome-wide RNA-mediated interference screen identifies miR-19 targets in Notch-induced T-cell acute lymphoblastic leukaemia. *Nat. Cell Biol.* 12, 372–379

83 Mu, P. *et al.* (2009) Genetic dissection of the miR-17~92 cluster of microRNAs in Myc-induced B-cell lymphomas. *Genes Dev.* 23, 2806–2811

84 Calin, G.A. *et al.* (2002) Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc. Natl. Acad. Sci. U.S.A.* 99, 15524–15529

85 Garzon, R. *et al.* (2009) MicroRNA 29b functions in acute myeloid leukemia. *Blood* 114, 5331–5341