Review

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MicroRNAs in cancer: biomarkers, functions and therapy

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The emergence of microRNAs has been one of the defining developments in cancer biology over the past decade, and the explosion of knowledge in this area has brought forward new diagnostic and therapeutic opportunities. The importance of microRNAs in cancer has been underlined by the identification of alterations in microRNA target binding sites and the microRNA processing machinery in tumor cells. Clinical trials utilizing microRNA profiling for patient prognosis and clinical response are now underway, and the first microRNA mimic entered the clinic for cancer therapy in 2013. In this article we review the potential applications of microRNAs for the clinical assessment of patient outcome in cancer, as well as in cancer monitoring and therapy.

MicroRNAs in cancer

In just a few short years microRNAs have become firmly established as key molecular components of the cell in both normal and pathologic states [1]. Cancer in particular has been a major focus of microRNA research over the past decade, and many studies have demonstrated the importance of microRNAs in cancer biology through controlling expression of their target mRNAs to facilitate tumor growth, invasion, angiogenesis, and immune evasion [2,3]. Additionally, tumor microRNA profiles can define relevant subtypes, patient survival, and treatment response [4–6]. Importantly, cancer-associated microRNA biomarkers can be detected in biological fluids, allowing less-invasive monitoring [7]. This review is timely because a number of clinical trials utilizing microRNA profiling for patient prognosis and clinical response are now underway, and the first microRNA mimic entered the clinic for cancer therapy last year [8]. Here, we summarize recent advances in the identification and characterization of microRNAs that may be used to facilitate patient diagnosis, prognosis, monitoring, and treatment in the oncology field.

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MicroRNA basics: discovery, biogenesis and mechanism

After their initial discovery in 1993 in studies on *Caenorhabditis elegans* [9], it was quickly recognized that microRNAs have a conserved mechanism and broad functional significance throughout the plant and animal kingdoms. Now there are over 2,500 potential human microRNAs recorded in miRBase (version 20, accessed January 2014), a searchable database of published microRNAs and their annotation [10]. The predominant function of microRNAs is to regulate protein translation by binding to complementary sequences in the 3′ untranslated region (UTR) of target messenger RNAs (mRNAs), and thereby negatively regulate mRNA translation [11]. The biogenesis of microRNAs generally occurs by a well-characterized conserved processing mechanism, which is outlined in Figure 1. MicroRNAs are encoded in the genome in various contexts: they can be expressed from intronic or intergenic transcripts, which may encode a single microRNA hairpin precursor, or clusters of multiple precursors. After processing, the mature single-stranded microRNAs, associated with Argonaute 2 (AGO2) in the RNA-induced silencing complex (RISC), typically bind to the 3′UTRs of their cytosolic mRNA targets, resulting in either reduced translation or deadenylation and degradation of the mRNA transcript. The fate of the mRNA depends on the degree of base-pairing complementarity between the mRNA molecule and the 'seed' region at the 5′ end of the microRNA [11]. The microRNA–mRNA binding site is short (6–8 base pairs), and therefore each microRNA has the potential to target multiple different mRNAs.

MicroRNAs in cancer: functions, alterations, and mechanisms

MicroRNA dysregulation in cancer was first reported in 2002, when a cluster of two microRNAs, miR-15 and miR-16, was identified at 13q14.3, a frequently deleted region in chronic lymphocytic leukemia (CLL) [12]. This microRNA deletion was shown to act at least in part through allowing higher expression of the miR-15/16 anti-apoptotic target B-cell lymphoma 2 (BCL2). Since then it has been documented that microRNAs have roles in all of the cancer hallmarks defined by Hanahan and Weinberg [13], and are implicated in the clinical management of cancers at every stage (Figure 2).

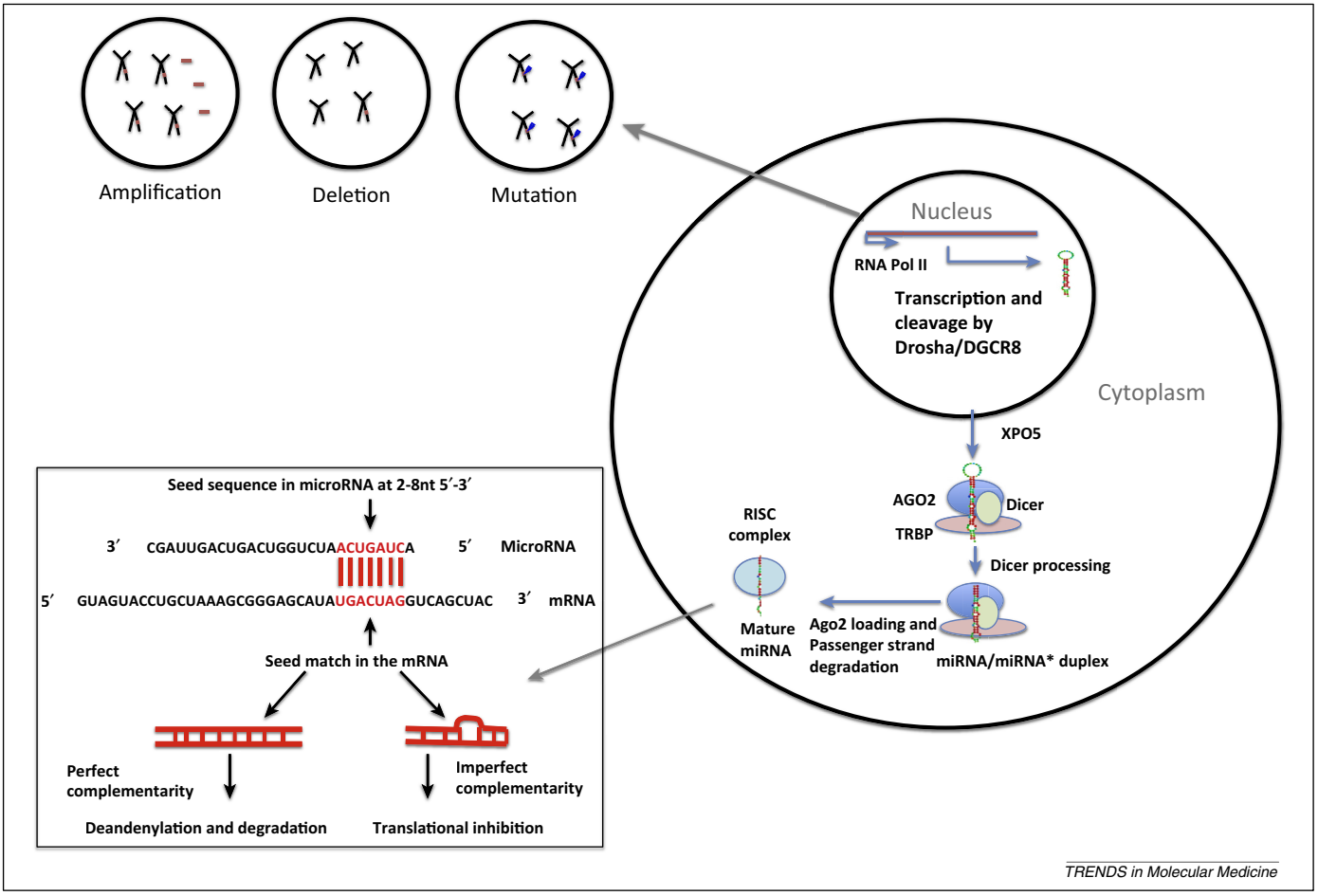

Figure 1. Biogenesis and function of microRNAs, and their dysregulation in cancer. MicroRNAs are transcribed by RNA polymerase II. The resulting primary microRNA transcript is cleaved in the nucleus by a complex involving Drosha and DGCR8 microprocessor complex subunit (DGCR8), forming what is termed a precursor microRNA [11]. This is then exported into the cytoplasm by exportin-5 (XPO5) [18], where it is cleaved by a multiprotein complex including the RNase Dicer, AGO2 (Argonaute 2), and TRBP (trans-activation-responsive RNA-binding protein) to form mature microRNA sequences. One part of the duplex is usually more abundant than the other, and the less abundant of the two is termed the 'star' or 'passenger' strand (miRNA*). These mature forms, incorporated into the RNA-induced silencing complex (RISC), then bind, with a 6mer to 8mer seed sequence, to the 3′UTR (and in some cases the coding sequence) of an mRNA molecule. The nature of this binding determines the fate of the microRNA, with perfect complementarity targeting the mRNA transcript for degradation, and imperfect complementarity inhibiting translation [11]. The dysregulation of expression of microRNAs in cancer can occur through multiple mechanisms. At the genomic level this can be due to amplification or deletion of the sequence, or mutations can alter the target site or the processing outcome of the microRNA.

Although many specific examples have been reported, microRNA functions fall into two broad major functional categories: (i) homeostatic regulation of gene expression, through 'fine-tuning' of translation according to cellular requirements; and (ii) robustness in cellular responses, which is important in cell fate decisions in which groups of microRNAs can dictate the cellular differentiation state, acting as 'locks' to maintain cell identity, often via complex reciprocal negative-feedback loops [1]. This is seen in some cancers, in which microRNAs associated with terminal differentiation are weakly expressed in order to promote a proliferative state [14]. Robustness is also important in responses to stress, in which microRNAs may function as 'switches' to allow cells to adapt to transient changes in their microenvironment. For example, in glioblastoma, low levels of glucose lead to a reduction in levels of miR-451, which is necessary for AMP-activated protein kinase (AMPK) pathway activation and cell survival. Conversely, when energy levels are sufficient, higher levels of miR-451 act to suppress AMPK signaling, and to promote mammalian target of rapamycin (mTOR) activation and cell proliferation [15].

Importantly, each tumor type has a distinct microRNA signature that distinguishes it from normal tissues and other cancer types. Most cancers can be further subclassified into prognostic groups based on these signatures [4]. MicroRNA expression, like the expression of other cancer-associated genes, can be altered by chromosomal amplification/deletion, promoter methylation, and transcription factor activation (Figure 1). The best-characterized cancer-associated microRNAs and their targets are described in Table 1. The importance of microRNA alterations in cancer is further highlighted by the observation that many cancer cells have genetic alterations that are microRNA mechanism-specific: that is, altered target binding, processing, and post-transcriptional editing. Binding site variation in the 3′UTR of the target mRNA is a common feature of cancer cells [16]. Single nucleotide polymorphisms (SNPs) and mutations have been identified, as well as deletions of 3′UTRs during mRNA splicing in cancer cells, rendering mRNAs insensitive to microRNA regulation [17].

Alterations in the microRNA processing machinery [18], and a global reduction of microRNAs in cancer cells

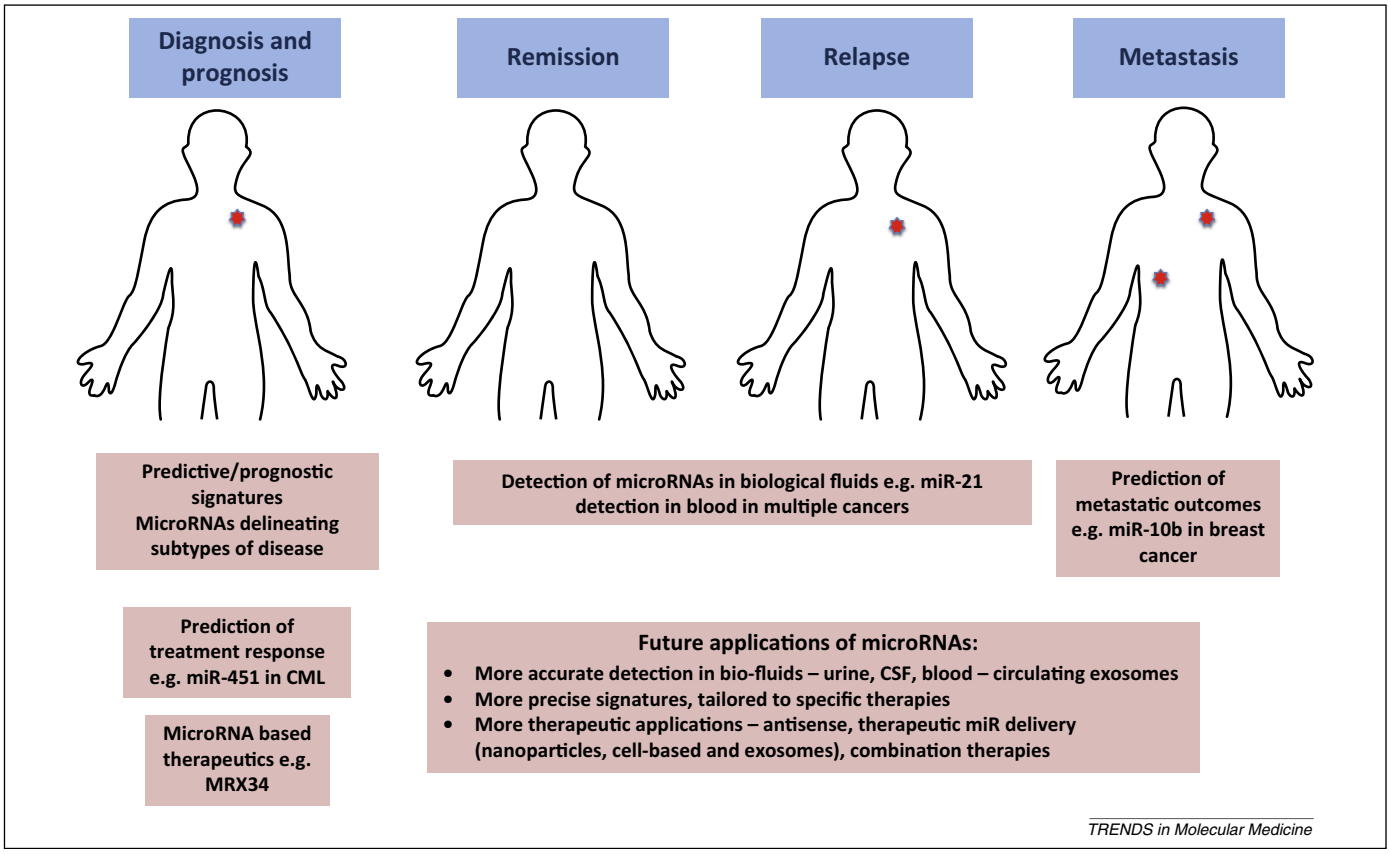

Figure 2. The impact of microRNAs in the clinical management of cancer. Due to the fact that microRNA expression patterns are altered in cancer compared to normal tissue, and also altered between subtypes, they are likely to be future biomarkers for the stratification and prediction of prognosis for cancer patients. In future cancer therapy, microRNAs may be important in deciding which drugs are selected for a patient, and in determination of whether the patient has responded to the drug. Monitoring of microRNA expression levels may determine whether relapse is imminent, and may also predict whether metastasis has occurred or is likely [55].

compared with normal tissue have been frequently reported [4]. Mutations that reduce the efficiency of the microRNA processing machinery have been identified, and these mutations affect the levels of mature microRNAs in the cell (Figure 3). For example, in some cancers with microsatellite instability, mutations in exportin-5 (*XPO5*), lead to trapping of pre-microRNAs in the nucleus, preventing further microRNA processing [18]. Also, reduced expression of Dicer predicts poorer outcome in ovarian cancer and contributes to drug resistance [19], consistent with the general assumption that global reduction of mature microRNAs is associated with poorer outcome [4]. The secondary structure of the pre-microRNA has been shown to be important for precise processing, and alterations of the loop structures in pre-microRNA sequences by mutations, SNPs, deletions, and duplications may prevent their efficient processing [20]. Nuclear export of pre-microRNAs has been reported to increase following radiation- or chemotherapy-induced DNA damage in HCT116 cells [21], where increased microRNA processing may maintain the cellular DNA damage response.

A less-well-explored area in cancer is the recognition of primary microRNA hairpins among a background of other hairpin RNAs, which is required for cleavage and nuclear export. Sequence determinants within the primary transcripts that license the hairpin for processing allow the binding of certain proteins, such as SRp20 [22]. Similarly, altered processing can occur by binding of BCDIN3D (bicoid-interacting 3, domain-containing), which O-methylates the 5′ monophosphate required by Dicer for efficient cleavage and therefore negatively regulates microRNA processing [23]. It is likely that alterations in these processes may also contribute to microRNA dysregulation in cancer.

MicroRNA action is also influenced by post-transcriptional editing, catalyzed by adenosine deaminases that act on RNA (ADARs), which convert adenine to inosine, inducing changes in target recognition through base-pairing with cytidine. Editing of miR-376* (the passenger strand of miR-376) has been reported in high-grade glioma and might affect patient outcome [24]. The increasing use of next-generation sequencing (NGS) and the efforts of the Cancer Genome Atlas (TCGA; http://tcga-data.nci.nih.gov/) [24] to provide large volumes of data are likely to highlight more examples such as this in the near future. Links between the editing machinery and the microRNA processing machinery are evident, suggesting that the balance between Dicer–ADAR1 complexes and ADAR1–ADAR1 homodimers dictates the levels of editing and microRNA processing in the cell [25].

Competing endogenous RNAs (ceRNAs) are RNA sequences comprising multiple microRNA binding sites, which act competitively to prevent the action of microRNAs on their mRNA targets by redirecting the RISC complex. This is an interesting case of nature imitating science, because before the discovery of ceRNAs, synthetic RNA

Table 1. Well-characterized microRNAs and their validated targets in cancer according to Tarbase [79]

MicroRNA	Mechanism		Targets
miR-17~92	Oncogene/tumor suppressor gene		E2F1, HBP1, CDKN1A, NCOA3, ERa, PTEN, MECP2, HOXA5, VPS4B, MYCN, RAB14, DPYSL2, TGFBR2, TSG101, ARHGAP12, BACE1,
miR-21	Oncogene	✓	PDCD4, PTEN, RECK, PPARa, TIMP3, FasL, TGFBR2, SERINB5, CDK2AP1, TPM1
miR-221/222	Oncogene	✓	CDKN1B, KIT, PPP2R2A, p27kip1, CDKN1C, ERa, KIT, DDIT4, BNIP3L, ZEB2, TBK1, CREBZF, MYBL1, DKK2
let-7	Tumor suppressor gene	✓	NIRF, NF2, CASP3, TRIM71
miR-15/16	Tumor suppressor gene		BACE1, DMTF1, C22orf5, BCL2, ARL2, CCNT2, TPPP3, VEGFA, RARS, FGF2, ZNF622, DNAJB4, PURA, SHOC2, LUZP1, FNDC3B, ITGA2, ATG9A, CA12, TMEM43, YIF1B, TMEM189, VTI1B, RTN4, TOMM34, NAA15, PNP, SRPR, IPO4, NAPg, PFAH1B2, SLC12A2, SEC24A, NOTCH2, PPP2R5C, KCNN4, UBE4A, KPNA3, RAB30, ACP2, SRPRB, EIF4E, ABCF2, TPM3, ARHGDIA, GALNT7, LYPLA2, CHORDC1, TMEM109, LAMC1, EGFR, GPAM, ADSS, PPIF, RFT1, TNFSF9, IGF2R, TXN2, GFPT1, SLC7A1, SQSTM1, PANX1, UTP15, NPR3, SLC16A3, PTGS2, HARS, LAMTOR3, HSPA1B
miR-200	Tumor suppressor gene	✓	ZEB1, CTNNB1, BAP1, GEMIN2, PTPRD, WDR37, KLF11, SEPT9, HOXB5, ERBB2IP. KLHL20, FOG2, RIN2, RASSF2, ELMO2, TCF7L1, VAC14, SHC1, SEPT7, FOG2
miR-34	Tumor suppressor gene	✓	SIRT1, BCL2, YY1, MYC, CDK6, CCND1, FOXP1, HNF4a, CDKN2C, ACSL4, LEF1, ACSL1, MTA2, AXL, LDHA, HDAC1, CD44, BCL2, E2F3

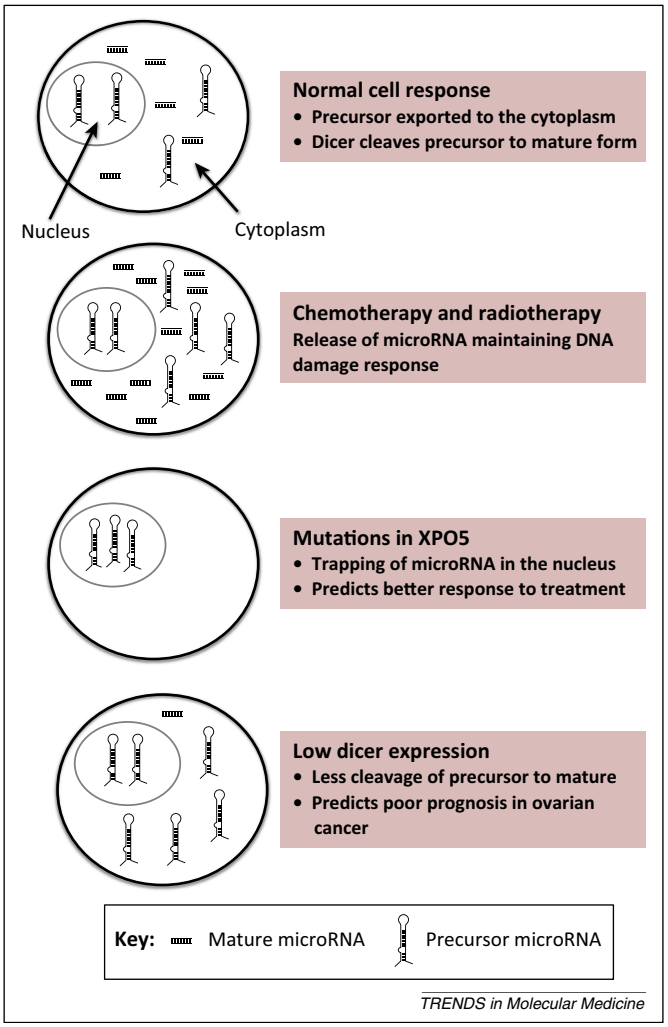

Figure 3. Alterations in the microRNA processing machinery observed in cancer. The normal processing of a microRNA requires transcription of a primary transcript, cleavage, exportation to the cytoplasm, and further cleavage to generate a mature transcript. If any of the machinery performing these steps is altered in cancer, there is global dysregulation of microRNAs in the cell. It has been shown that chemo- and radiotherapy cause an increase in microRNAs in the cell, which acts to maintain the DNA damage response [21]. Mutations in the exportation machinery, such as exportin-5 (XPO5), lead to a build-up of precursor microRNA in the nucleus and a lack of mature microRNAs in the cytoplasm [18], where they usually exert their function. Low expression of processing components, such as Dicer, can dramatically reduce the numbers of microRNAs cleaved from precursor to mature form, again reducing their downstream effects on the cell [19]. Lower Dicer expression also predicts poor prognosis in ovarian cancer [19].

sequences termed microRNA sponges were already being used to block specific microRNAs [26]. ceRNAs include long non-coding RNAs (lncRNAs), pseudogenes, circular RNAs, and, in some cases, protein-coding mRNAs. For example, levels of phosphatase and tensin homolog (PTEN) are regulated in cancer cells by multiple ceRNAs, which influence the availability of microRNAs that target PTEN expression [27]. A circular RNA, CDR1 (cerebellar degeneration-related protein 1), contains over 60 binding sites for miR-7 [28], a tumor suppressor microRNA with multiple oncogenic targets [29], and the mRNA encoding HMGA2 (high mobility group AT-hook 2) acts as a ceRNA for the let-7 family, promoting lung cancer progression [30].

MicroRNA networks in cancer

The presence at any given time of ceRNAs and multiple sequences in the cell that may 'compete' for microRNA binding emphasizes the extensive networks that are involved in microRNA function. The imperfect match and relatively short 6–8 base pair seed sequence characteristic of microRNA–mRNA interactions allow for a multitude of potential targets for each microRNA. Additionally, a single mRNA may have target sites for multiple microRNAs, creating redundant molecular networks for the control of gene expression (Figure 1 and Box 1). As a result of the potential to predict microRNA-binding sites based on base pairing, they are highly amenable to systems biology approaches. However, many studies have focused narrowly on the specific effect of a given microRNA on a specific mRNA, defined by bioinformatic prediction algorithms, rather than exploring the 'bigger picture' of gene expression regulation as part of an extended network [31]. One of the reasons for the focus on single bioinformatically predicted targets is experimental tractability, because it is not trivial to identify microRNA targets experimentally in mammalian cells, and many important interactions have been identified using this method. However, the microRNA/single mRNA target approach can lead to 'cherry

Box 1. MicroRNA networks

Reviewing the microRNA literature on cancer, it is possible to discern at least four different philosophical approaches to the study of these small RNAs (Figure I).

A: The basic approach: single microRNA–mRNA interaction
This approach has helped define some of the most relevant microRNAs in cancer, but ultimately offers little more information than just confirming the repressive action of one microRNA over one predicted target. The value of such information relies on the magnitude of the biological effect that ensues, and in the ability to build-up over time a 'catalog' of microRNAs and their direct targets (see Table 1 in main text). Such an approach is based on the assumption that microRNAs are like any other protein-coding genes, and not, as we believe, the most versatile instrument that each cell has to regulate and coordinate its multiple, often opposing, molecular pathways, by virtue of their multi-targeting ability.

B: The broad approach: one microRNA to many mRNAs
More in-depth studies have sought to identify a number of targets for the same microRNAs that would result in a sum of effects determining a common phenotype.

C: The functional approach
A step further in complexity, a microRNA not only targets transcripts that have a similar effect to a cell's biology, but they are also functionally related, in the sense that they interact/regulate with each other to obtain their biological effect.

D: The pathway-based approach
In this scenario, one microRNA or multiple, related microRNAs (such as those belonging to clusters, and thus regulated by similar transcription factors (TFs)) target several proteins within a defined pathway inducing a profound regulation of that pathway at several nodal points, including the final effect.

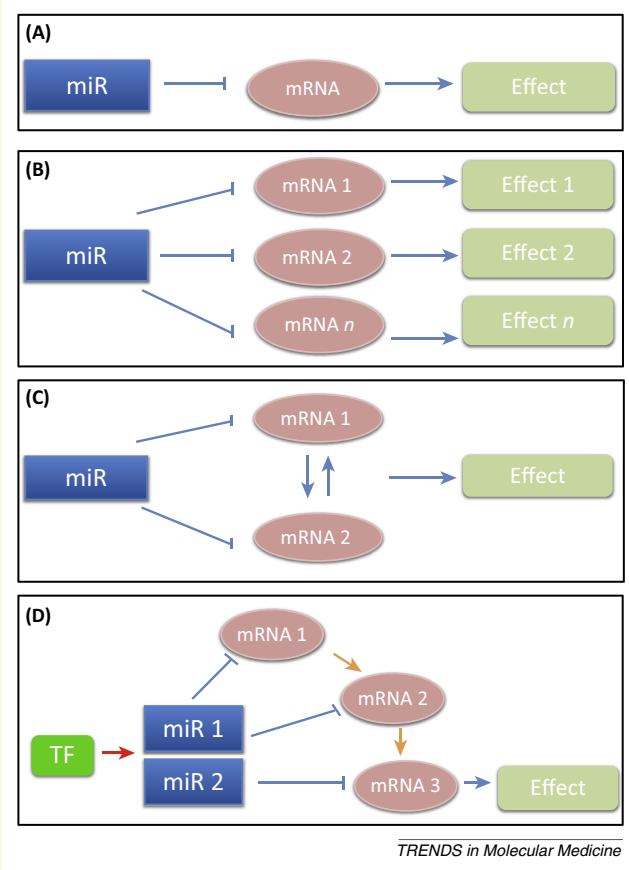

Figure I. Approaches to studying microRNA networks. **(A)** The 'basic' approach: single microRNA–mRNA interaction. **(B)** The 'broad' approach: one microRNA to many mRNAs. **(C)** The 'functional' approach. **(D)** The pathway-based approach.

picking' of specific targets that might not accurately reflect the most physiologically significant microRNA–target interactions. This can be overcome by screening multiple targets, or using global approaches to identify microRNA–target interactions inside the cell; these approaches include proteomics, gene expression arrays, and RNA cross-linking/AGO2 pull-down approaches such as HITS-CLIP (high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation) to allow assessment of microRNA–target binding in the cell [32–34]. Future studies in the field would benefit from the application of these techniques, as well as the assessment of microRNA functions in the context of networks, including sponge interactions and feedback loops that take into account the competitive nature of interactions between microRNAs and their targets.

The influence of groups of microRNAs is exemplified by microRNAs in clusters, which are expressed together and show functional cooperation. For example, the polycistronic oncogenic miR-17~92 cluster of microRNAs specifically induces lymphomagenesis in a B-cell-specific transgenic mouse model [35], and miR-19b, miR-20a, and miR-92 from this cluster, along with miR-26a and miR-223, promote T-cell acute lymphoblastic leukemia (ALL) development in mouse models [36].

Intercellular network interactions should also be addressed, as shown by the symmetry of cell division in colorectal cancer (CRC). In late-stage CRC and CRC stem cells, divisions are symmetric and produce two self-renewing daughter cells. In early-stage CRC, cell-fate determinants are localized to opposite poles during division, resulting in one self-renewing and one differentiating daughter cell (asymmetric cell division). This has been shown to be controlled by a Snail/miR-146a–Numb–β-catenin axis [37]. This suggests that miR-146a, induced through Snail-dependent β-catenin and TCF, downregulates Numb, relieving Numb-mediated degradation of β-catenin and subsequently enhancing Wnt signaling [38]. This effect maintains self-renewing divisions, partly independent of the epithelial–mesenchymal transition (EMT).

As part of their role in shaping the fate of a cell, microRNAs are fundamental in the control of EMT. Some microRNAs, such as the miR-200 family and miR-34a, are protectors of the epithelial phenotype, and their down-regulation during EMT enhances mesenchymal-specifying targets such as ZEB1 (zinc finger E-box binding homeobox 1) and ZEB2 [39]. The miR-34a family can also be inhibited by ZEB1 [40], establishing a robust feedback loop to ensure the cell is driven towards a more mesenchymal fate. Oncogenic microRNA miR-22 has been shown to

Table 2. Current open cancer related clinical trials with a significant microRNA component (ClinicalTrials.gov)

MicroRNA	Trial reference	Disease	Trial
miR-34a	NCT01829971	Liver cancer and liver metastases	Interventional phase I multicenter study to investigate the safety, pharmacokinetics and pharmacodynamics of MRX34
Numerous	NCT01964508	Thyroid cancer	Observational study of microRNA expression in fine needle aspirates
Circulating	NCT01722851	Breast cancer	Observational studies of microRNA expression levels as biomarkers of response to treatment in the tumors and circulation
Numerous	NCT01220427	High-risk prostate cancer	Observational studies of microRNA expression levels as biomarkers of response to treatment in the tumors
miR-10b	NCT01849952	Glioma	Observational studies of miR-10b expression levels as biomarkers of tumor grade, survival, and genotypic variation
miR-29b	NCT02009852	Oral squamous cell carcinoma	Observational study to explore the prognostic value of miR-29b in tissue, blood, and saliva
Numerous	NCT02127073	Breast cancer	Interventional study employing intranasal oxytocin to increase the volume of nipple aspirate fluid for biomarker identification and subsequent microRNA profiling of this aspirate
Circulating	NCT01595139	Low-grade glioma	Observational study of the circulating microRNA expression patterns in low grade glioma as early predictors of cancer and as a marker of response to therapy
Numerous	NCT01828918	Colorectal carcinoma	Observational study identifying biomarkers for patient stratification in tissue samples
Numerous	NCT01119573	Endometrial cancer	Observational study of association of microRNA expression and lymph node metastasis in tissue samples
Circulating	NCT01595126	Central nervous system cancer	Observational study of microRNA expression in the blood, cerebrospinal fluid and urine of patients through the course of their treatment
miR-29 family	NCT01927354	Head and neck squamous cell carcinoma	Observational study to investigate the role of microRNA in Twist1-mediated cancer metastasis
Numerous	NCT01453465	Rhabdoid tumors	Observational study to identify microRNA expression patterns between rhabdoid tumors of the kidney and atypical teratoid rhabdoid tumors
Circulating	NCT01391351	Ovarian cancer	Observational studies of biomarker of response to treatment
Circulating	NCT01505699	B-cell acute lymphocytic leukemia	Observational studies of biomarkers of clinical outcomes
Numerous	NCT01957332	Breast cancer	Observational study correlating microRNA expression patterns with imaging and clinical data
Circulating	NCT01556178	Pediatric brain cancer	Observational study of microRNAs in the blood and cerebrospinal fluid as biomarkers
Numerous	NCT01050296	Pediatric solid tumors	Observational study of microRNA expression profiles in different tumor types
Numerous	NCT00864266	Non-small-cell lung cancer	Interventional study to identify a signature of response to chemotherapy
Numerous	NCT01108159	Hematologic cancer	Observational studies of biomarker of expression profiles in initiation, progression and treatment response

inhibit anti-metastatic miR-200 in breast cancer by targeting the ten eleven translocation (TET) family of methylcytosine dioxygenases, which results in silencing of miR-200 [41]. Positive correlation of miR-138 and EMT has uncovered its role in driving the process through multiple targets including Vimentin, transcriptional repressors such as ZEB2, and epigenetic regulators such as EZH2 (enhancer of zeste homolog 2) [42]. Similarly, miR-155 has been shown to repress transforming growth factor-β (TGF-β)-induced EMT, and depletion of this microRNA can suppress EMT in a mouse model [43].

The study of microRNAs under stress conditions has uncovered some important findings, including the epidermal growth factor receptor (EGFR)-mediated phosphorylation of AGO2 in response to hypoxia in breast cancer, resulting in suppression of specific microRNAs that depend on AGO2 for their maturation [44]. This is of huge importance in cancers in which EGFR is constitutively active. Our increasing knowledge of microRNA regulatory networks has underlined the importance of microRNA control over tumor cell biology, and has highlighted novel therapeutic targets and processes involved in tumor growth. One such example of this is the recent discovery that miR-542-3p weakens the interaction of p53 with its negative regulator mouse double minute 2 homolog (MDM2), thus stabilizing the protein [45].

MicroRNAs as predictors of prognosis

Our progressively greater understanding of the molecular alterations underlying cancer has created opportunities for more accurate and meaningful diagnosis and prognosis than was previously possible. Now, entering into the era of personalized medicine, patient-management decisions increasingly depend on molecular analyses. In addition to specific genetic alterations, additional molecular features including DNA methylation, gene expression, and microRNA expression can provide vital clinical information. MicroRNAs may have greater utility than mRNAs as prognostic indicators owing to their stability within clinical samples and their robust expression patterns [4]. Many signatures have been proposed, and their use is being investigated in clinical trials (Table 2). Until fairly recently, microRNA analysis was performed using qRT-PCR and microarray-based approaches. NGS has emerged as a cost-effective option, and the barriers to bioinformatics analysis are no longer a daunting prospect to the non-specialist laboratory [46].

A meta-analysis of 43 studies in 20 cancer types highlighted some of the variability in studies of microRNA

signatures, which can occur for many reasons, including sample preparation, assay methodology, and patient characteristics [47]. Of all microRNAs, miR-21 (increased in cancer) and let-7 (decreased in cancer) were the most common microRNAs associated with patient outcome. The oncogenic miR-21 is overexpressed in many cancers, including breast cancer, glioblastoma, hepatocellular carcinoma, lung cancer, stomach cancer, colorectal cancer, and prostate cancer [48]. It creates a pro-tumorigenic environment by targeting numerous tumor suppressor genes with roles in apoptosis, invasion, and proliferation [48], and so has emerged as a novel molecular target for cancer therapy.

MicroRNAs for classification of disease

MicroRNA expression signatures often reflect embryonic or developmental origin of the tumor type [4]. This greatly facilitates tumor classification based on microRNAs: a blind study of 22 different tumor types showed microRNA expression classified tumors according to tissue of origin with accuracy higher than 90% [49]. WHO classification of leukemia is done according to progenitor cell lineage [50], and microRNA expression across leukemia subtypes also reflects the cell of origin. Association of expression signatures in acute myeloid leukemia (AML) revealed specific microRNA expression patterns in each disease subtype [51]. Similarly, in prostate cancer, microRNA patterns were distinct between different cellular subsets when stem/progenitor cells were isolated from prostate tumors, showing that microRNA expression patterns are indicative of the cellular populations in a tumor [52]. Distinct microRNA expression patterns have also recently been identified in luminal (epithelial origin), basal-like (myoepithelial origin), and human epidermal growth factor receptor 2 (HER2) breast cancers [53]. The classification of breast cancer is better defined that most malignancies; however, meta-analyses of recent clinical trials have shown incorrect classification of a substantial number of tumors in laboratories with high volume testing (fluorescence *in situ* hybridization and immunohistochemistry-based tests) [54], and therefore microRNA analysis may add robustness to current testing.

Various cancer subtypes have been identified using the huge body of data at TCGA, and microRNAs are either associated with these changes or have been used to create subtypes [5]. The identification of microRNAs that target current biomarkers may pave the way for microRNA-based tests as an alternative to mRNA/protein expression for prognosis assessment. MicroRNAs have been shown to have a role in cancer progression [55] and might be useful for the prediction of metastatic outcomes for patient management. Specific microRNAs have been shown to support endothelial recruitment to metastases in breast cancer and might serve as efficient biomarkers for predicting this event [56]. MicroRNA signatures associated with known inducers of EMT have also been developed and shown to be relevant in both *in vitro* and *in vivo* models of EMT in endometrial cancer [57].

MicroRNAs as predictors of drug efficacy

Although not yet used in clinical decision making, several studies have associated microRNAs with well-known biomarkers for treatment therapy decisions. For example, in chronic myeloid leukemia (CML), levels of cells with the BCR-ABL rearrangement, which characterizes this disease, decrease over time with imatinib treatment. It has been discovered that miR-451 levels inversely correlate with BCR-ABL levels at both the time of diagnosis and on treatment [58]. SNPs in microRNA target sites may also be predictors of response; the LCS6 polymorphism in the let-7 binding site in the 3′UTR of KRAS predicted response to anti-EGFR-based therapy in 100 metastatic CRC cancer patients [59]. Base excision repair genes have been associated with treatment resistance, and variations in the microRNA binding sites of the 3′UTRs of these genes have been shown to reflect CRC cancer prognosis and treatment response [60]. A notable and interesting example of altered target sites in cancer is the creation of an illegitimate target site for miR-191 in the 3′UTR of MDM4 by the presence of SNP34091, which affects chemosensitivity in ovarian cancer [61].

MicroRNA polymorphisms predisposing cancer

Aside from treatment resistance, it is worth noting that a number of SNPs in microRNA binding sites are involved in cancer risk and may be markers for genetic susceptibility studies in some cancers [16]. They can be used as markers to predict subsets of patients at risk of poor outcome or lack of treatment response. These SNPs may be present in microRNA target sites, in the processing machinery, or in the microRNA sequence, altering the target of the microRNA and its ability to be processed [62–64].

MicroRNAs as non-invasive biomarkers

The use of circulating microRNAs as markers in different cancer types is a rapidly developing area [65]. Tumor cells can release microRNAs, stabilized by their incorporation into microvesicles, which have shown stability in the circulation following multiple freeze–thaw cycles and prolonged exposure to room temperature [66]. MicroRNAs have also shown stability in other bodily fluids, such as urine and saliva [65]; however, most studies have centered around serum microRNAs as biomarkers. A study of 391 patients with non-small cell lung cancer (NSCLC) identified 35 highly expressed microRNAs with predicted binding sites for at least one of 11 genes of the TGF-β pathway, which were significantly differentially expressed at the extremes of survival. Of these, 17 were associated with patient survival and were combined into a risk score that significantly predicted survival in advanced NSCLC [67]. Furthermore, isolation of exosomes from serum showed that a signature involving two microRNAs and one small non-coding RNA can be used for non-invasive diagnosis of glioblastoma [7].

The detection of microRNAs in the blood presents some challenges, and there is an overwhelming discordance between reports in well-studied cancers [68]. Appropriate endogenous controls for microRNA quantification in serum are under debate because many mRNA and rRNA species are absent in blood due to circulating RNases [69]. Clinically, fluctuations of circulating microRNAs can occur as a result of treatment, diet, and other factors, increasing noise in these assays. The presence of myeloid and

lymphoid cells can alter the levels of certain microRNAs, and viral infections of the patient might also affect endogenous microRNA expression [70]. Expression changes of microRNAs are rapid in blood, and even a traumatic venepuncture may have the potential to influence expression. Despite these hurdles, it is clear that further study is warranted for detection of the presence of microRNAs in the blood for future non-invasive biomarker development, and the field is moving rapidly towards that goal.

MicroRNA-based therapeutics and clinical trials

Most current clinical trials are for the use of microRNAs as biomarkers for patient stratification, prognosis, and drug efficacy, and breast cancer seems to be the cancer under the most study. In few cases are specific microRNAs stated, and a global expression-profiling platform has been employed for the mining of appropriate biomarkers. In addition to biomarker studies, microRNAs and anti-microRNA constructs are now under investigation as potential therapeutic agents for cancer. Despite the challenges presented by delivery of these types of molecule, there are currently two clinical trials for microRNA-based therapeutics [8] (Table 2). Targeting microRNAs may be used directly to target tumor cells, and also to enhance other therapies, for example, they may have a potential use in reducing the drug resistance of tumors as has been shown by the chemo-resistant properties of miR-100 in small cell lung cancer [71] and the epigenetic silencing of miR-199b-5p in chemoresistant ovarian cancer [72].

The most advanced microRNA trial involves use of anti-miR-122 (Miravirsen) for hepatitis C therapy [73], which shows reduction in viral RNA with no evidence of resistance. Miravirsen is complementary in sequence to miR-122 but also has a modified locked-nucleic acid structure, which provides resistance to degradation and increased affinity for its target. More recent studies have shown that although the intended target of Miravirsen is mature miR-122, it also has affinity for pri- and pre-miR-122 leading to reduced processing [74] and enhancement of its therapeutic effect.

The first microRNA-based therapy specifically for cancer is MRX34: a synthetic miR-34a mimic loaded in liposomal nanoparticles [8]. miR-34a is a tumor suppressor microRNA downstream of p53. Its replacement in cancer cells antagonizes key hallmarks including self-renewal, migratory potential, and chemo-resistance [75]. MRX34 is in a phase I clinical trial for primary liver cancer and liver metastases and should complete by the end of 2014. MRX34 nanoparticles readily accumulate in the liver, and quantification of MRX34 in non-human primates has established a satisfactory 7.7 h half-life in whole blood [76]. Lipid-based local and systemic delivery of miR-34a in animal studies has also shown positive results for lung cancer [77].

For microRNA-based therapeutics, resistance may become a factor, which may be overcome by using combinatorial microRNA-based therapies. With similar effect, certain anti-microRNA therapies have the potential to target whole families of microRNAs [78], reducing the likelihood of resistance. The study of microRNA-based therapies is still in its infancy, and side effects of these therapies need to be evaluated. MicroRNAs have been shown to be exported from cells in exosomes [7] and therefore have the potential for systemic effects which might only become apparent in clinical trials. Also, the processing of other microRNAs is likely to be dampened by overloading the microRNA processing machinery with replacement microRNAs, and the effects of this are uncertain [2].

Concluding remarks

MicroRNAs offer an attractive option as stable biomarkers for cancer detection, diagnosis, and prognosis assessment in both the tumor tissue and circulation. It will be important to carry out prospective trials in well-defined, large patient groups, and it would also be important that particular microRNAs used in the signatures are better characterized functionally. Measures should be employed to ensure their stability during sample processing and standardized validation studies should be undertaken to establish their robustness. A number of candidate microRNA signatures are emerging, and clinical trials assessing some of these are underway. It is expected that microRNA alterations will be observed in tumor heterogeneity, and these alterations may be drivers of heterogeneity.

In reality, it is likely that a signature encompassing information on integrated clinico-pathological, mRNA, and microRNA data will be the most relevant. Few studies incorporate more than one of these levels, which reduces the power of both in-house and publically available data.

The literature on microRNAs in cancer has expanded at a remarkable rate since they were first linked with the disease in 2002, and it is becoming rapidly obvious that they have clinical utility for the prediction of prognosis in various diseases. In addition to microRNAs, it is beginning to emerge that there are other non-coding RNAs with as yet unknown functions, including long non-coding RNAs, small nucleolar RNAs, and circular RNAs, which may provide clues to cancer mechanisms as well as improve molecular diagnostics for personalized medicine in human cancer.

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References

1 Ebert, M.S. and Sharp, P.A. (2012) Roles for microRNAs in conferring robustness to biological processes. *Cell* 149, 515–524
2 Choudhury, Y. *et al.* (2012) Epigenetics and genetics. MicroRNAs en route to the clinic: progress in validating and targeting microRNAs for cancer therapy. *J. Clin. Invest.* 122, 4059–4076
3 Stahlhut, C. and Slack, F.J. (2013) MicroRNAs and the cancer phenotype: profiling, signatures and clinical implications. *Genome Med.* 5, 111
4 Lu, J. *et al.* (2005) MicroRNA expression profiles classify human cancers. *Nat Cell Biol* 435, 834–838
5 Dvinge, H. *et al.* (2013) The shaping and functional consequences of the microRNA landscape in breast cancer. *Nature* 497, 378–382
6 Kim, T-M. *et al.* (2011) A developmental taxonomy of glioblastoma defined and maintained by microRNAs. *Cancer Res.* 71, 3387–3399
7 Manterola, L. *et al.* (2014) A small noncoding RNA signature found in exosomes of GBM patient serum as a diagnostic tool. *Neuro Oncol.* 16, 520–527

8 Bouchie, A. (2013) First microRNA mimic enters clinic. *Nat. Biotechnol.* 31, 577
9 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
10 Griffiths-Jones, S. *et al.* (2008) miRBase: tools for microRNA genomics. *Nucleic Acids Res.* 36, D154–D158
11 Krol, J. *et al.* (2010) The widespread regulation of microRNA biogenesis, function and decay. *Nat. Rev. Genet.* 11, 597–610
12 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
13 Hanahan, D. and Weinberg, R.A. (2011) Hallmarks of cancer: the next generation. *Cell* 144, 646–674
14 Chiocca, E.A. and Lawler, S.E. (2010) The many functions of microRNAs in glioblastoma. *World Neurosurg.* 73, 598–601
15 Godlewski, J. *et al.* (2010) microRNA-451: a conditional switch controlling glioma cell proliferation and migration. *Cell Cycle* 9, 2742–2748
16 Ziebarth, J.D. *et al.* (2012) Integrative analysis of somatic mutations altering microRNA targeting in cancer genomes. *PLoS ONE* 7, e47137
17 Sun, G. *et al.* (2009) SNPs in human miRNA genes affect biogenesis and function. *RNA* 15, 1640–1651
18 Melo, S.A. *et al.* (2010) A genetic defect in exportin-5 traps precursor microRNAs in the nucleus of cancer cells. *Cancer Cell* 18, 303–315
19 Kuang, Y. *et al.* (2013) Repression of Dicer is associated with invasive phenotype and chemoresistance in ovarian cancer. *Oncol. Lett.* 5, 1149–1154
20 Gu, S. *et al.* (2012) The loop position of shRNAs and pre-miRNAs is critical for the accuracy of dicer processing in vivo. *Cell* 151, 900–911
21 Wan, G. *et al.* (2013) DNA-damage-induced nuclear export of precursor microRNAs is regulated by the ATM-AKT pathway. *Cell Rep.* 3, 2100–2112
22 Auyeung, V.C. *et al.* (2013) Beyond secondary structure: primary-sequence determinants license pri-miRNA hairpins for processing. *Cell* 152, 844–858
23 Xhemalce, B. *et al.* (2012) Human RNA methyltransferase BCDIN3D regulates microRNA processing. *Cell* 151, 278–288
24 Choudhury, Y. *et al.* (2012) Attenuated adenosine-to-inosine editing of microRNA-376a* promotes invasiveness of glioblastoma cells. *J. Clin. Invest.* 122, 4059–4076
25 Ota, H. *et al.* (2013) ADAR1 forms a complex with Dicer to promote microRNA processing and RNA-induced gene silencing. *Cell* 153, 575–589
26 Ebert, M.S. *et al.* (2007) MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. *Nat. Methods* 4, 721–726
27 Tay, Y. *et al.* (2011) Coding-independent regulation of the tumor suppressor PTEN by competing endogenous mRNAs. *Cell* 147, 344–357
28 Memczak, S. *et al.* (2014) Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature* 495, 333–338
29 Hansen, T.B. *et al.* (2013) Circular RNA and miR-7 in Cancer. *Cancer Res.* 73, 5609–5612
30 Kumar, M.S. *et al.* (2014) HMGA2 functions as a competing endogenous RNA to promote lung cancer progression. *Nature* 505, 212–217
31 Sumazin, P. *et al.* (2011) An extensive microRNA-mediated network of RNA-RNA interactions regulates established oncogenic pathways in glioblastoma. *Cell* 147, 370–381
32 Chi, S.W. *et al.* (2009) Argonaute HITS-CLIP decodes microRNA-mRNA interaction maps. *Nature* 460, 479–486
33 Boudreau, R.L. *et al.* (2014) Transcriptome-wide discovery of microRNA binding sites in human brain. *Neuron* 81, 294–305
34 Hu, C-W. *et al.* (2013) Quantitative proteomics reveals diverse roles of miR-148a from gastric cancer progression to neurological development. *J. Proteome Res.* 12, 3993–4004
35 Sandhu, S.K. *et al.* (2013) B-cell malignancies in microRNA Emu-miR-17~92 transgenic mice. *Proc. Natl. Acad. Sci. U.S.A.* 110, 18208–18213
36 Mavrakis, K.J. *et al.* (2011) A cooperative microRNA-tumor suppressor gene network in acute T-cell lymphoblastic leukemia (T-ALL). *Nat. Genet.* 43, 673–678
37 Lerner, R.G. and Petritsch, C. (2014) A microRNA-operated switch of asymmetric-to-symmetric cancer stem cell divisions. *Nat. Cell Biol.* 16, 212–214
38 Hwang, W-L. *et al.* (2014) MicroRNA-146a directs the symmetric division of Snail-dominant colorectal cancer stem cells. *Nat. Cell Biol.* 16, 268–280
39 Hao, J. *et al.* (2014) MicroRNA control of epithelial-mesenchymal transition in cancer stem cells. *Int. J. Cancer* 135, 1019–1027
40 Kim, N.H. *et al.* (2011) A p53/miRNA-34 axis regulates Snail1-dependent cancer cell epithelial-mesenchymal transition. *J. Cell Biol.* 195, 417–433
41 Song, S.J. *et al.* (2013) MicroRNA-antagonism regulates breast cancer stemness and metastasis via TET-family-dependent chromatin remodeling. *Cell* 154, 311–324
42 Liu, X. *et al.* (2011) MicroRNA-138 suppresses epithelial-mesenchymal transition in squamous cell carcinoma cell lines. *Biochem. J.* 440, 23–31
43 Kong, W. *et al.* (2008) MicroRNA-155 is regulated by the transforming growth factor beta/Smad pathway and contributes to epithelial cell plasticity by targeting RhoA. *Mol. Cell. Biol.* 28, 6773–6784
44 Shen, J. *et al.* (2014) EGFR modulates microRNA maturation in response to hypoxia through phosphorylation of AGO2. *Nature* 497, 383–387
45 Wang, Y. *et al.* (2014) p53 is positively regulated by miR-542-3p. *Cancer Res.* 74, 3218–3227
46 Farazi, T.A. *et al.* (2012) Bioinformatic analysis of barcoded cDNA libraries for small RNA profiling by next-generation sequencing. *Methods* 58, 171–187
47 Nair, V.S. *et al.* (2012) Clinical outcome prediction by microRNAs in human cancer: a systematic review. *JNCI J. Natl. Cancer Inst.* 104, 528–540
48 Pan, X. *et al.* (2010) MicroRNA-21: a novel therapeutic target in human cancer. *Cancer Biol. Ther.* 10, 1224–1232
49 Rosenfeld, N. *et al.* (2008) MicroRNAs accurately identify cancer tissue origin. *Nat. Biotechnol.* 26, 462–469
50 Wandt, H. *et al.* (2010) WHO classification of myeloid neoplasms and leukemia. *Blood* 115, 748–749 author reply 749–750
51 Garzon, R. *et al.* (2008) MicroRNA signatures associated with cytogenetics and prognosis in acute myeloid leukemia. *Blood* 111, 3183–3189
52 Liu, C. *et al.* (2012) Distinct microRNA expression profiles in prostate cancer stem/progenitor cells and tumor-suppressive functions of let-7. *Cancer Res.* 72, 3393–3404
53 Farazi, T.A. *et al.* (2014) Identification of distinct miRNA target regulation between breast cancer molecular subtypes using AGO2-PAR-CLIP and patient datasets. *Genome Biol.* 15, R9
54 Andorfer, C.A. *et al.* (2011) MicroRNA signatures: clinicalbiomarkers for the diagnosis andtreatment of breast cancer. *Trends Mol. Med.* 17, 313–319
55 Pencheva, N. and Tavazoie, S.F. (2013) Control of metastatic progression by microRNA regulatory networks. *Nat. Cell Biol.* 15, 546–554
56 Png, K.J. *et al.* (2012) A microRNA regulon that mediates endothelial recruitment and metastasis by cancer cells. *Nature* 481, 190–194
57 Díaz-Martín, J. *et al.* (2014) A core microRNA signature associated with inducers of the epithelial-to-mesenchymal transition. *J. Pathol.* 232, 319–329
58 Scholl, V. *et al.* (2012) Leukemia research. *Leukemia Res.* 36, 119–121
59 Sebio, A. *et al.* (2013) The LCS6 polymorphism in the binding site of let-7 microRNA to the KRAS 3′-untranslated region: its role in the efficacy of anti-EGFR-based therapy in metastatic colorectal cancer patients. *Pharmacogenet. Genomics* 23, 142–147
60 Pardini, B. *et al.* (2013) Variation within 3′-UTRs of base excision repair genes and response to therapy in colorectal cancer patients: a potential modulation of microRNAs binding. *Clin. Cancer Res.* 19, 6044–6056
61 Wynendaele, J. *et al.* (2010) An illegitimate microRNA target site within the 3′ UTR of MDM4 affects ovarian cancer progression and chemosensitivity. *Cancer Res.* 70, 9641–9649
62 Ding, C. *et al.* (2013) A miR-SNP of the XPO5 gene is associated with advanced non-small-cell lung cancer. *Onco Targets Ther.* 6, 877–881
63 Wu, Y. *et al.* (2011) A miR-200b/200c/429-binding site polymorphism in the 3′ untranslated region of the AP-2alpha gene is associated with cisplatin resistance. *PLoS ONE* 6, e29043
64 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

65 Schwarzenbach, H. *et al.* (2014) Clinical relevance of circulating cell-free microRNAs in cancer. *Nat. Rev. Clin. Oncol.* 11, 145–156
66 Mitchell, P.S. *et al.* (2008) Circulating microRNAs as stable blood-based markers for cancer detection. *Proc. Natl. Acad. Sci. U.S.A.* 105, 10513–10518
67 Wang, Y. *et al.* (2013) Pathway-based serum microRNA profiling and survival in patients with advanced stage non-small cell lung cancer. *Cancer Res.* 73, 4801–4809
68 Jarry, J. *et al.* (2014) The validity of circulating microRNAs in oncology: five years of challenges and contradictions. *Mol. Oncol.* 8, 819–829
69 Li, Y. and Kowdley, K.V. (2012) Method for microRNA isolation from clinical serum samples. *Anal. Biochem.* 431, 69–75
70 Pritchard, C.C. *et al.* (2012) Blood cell origin of circulating microRNAs: a cautionary note for cancer biomarker studies. *Cancer Prev. Res. (Phila.)* 5, 492–497
71 Xiao, F. *et al.* (2014) Downregulation of HOXA1 gene affects small cell lung cancer cell survival and chemoresistance under the regulation of miR-100. *Eur. J. Cancer* 50, 1541–1554
72 Liu, M.X. *et al.* (2014) Epigenetic silencing of microRNA-199b-5p is associated with acquired chemoresistance via activation of JAG1-Notch1 signaling in ovarian cancer. *Oncotarget* 5, 944–958
73 Janssen, H.L.A. *et al.* (2013) Treatment of HCV infection by targeting microRNA. *N. Engl. J. Med.* 368, 1685–1694
74 Gebert, L.F.R. *et al.* (2014) Miravirsen (SPC3649) can inhibit the biogenesis of miR-122. *Nucleic Acids Res.* 42, 609–621
75 Bader, A.G. (2012) miR-34 – a microRNA replacement therapy is headed to the clinic. *Front. Genet.* 3, 120
76 Kelnar, K. *et al.* (2014) Quantification of therapeutic miRNA mimics in whole blood from nonhuman primates. *Anal. Chem.* 86, 1534–1542
77 Wiggins, J.F. *et al.* (2010) Development of a lung cancer therapeutic based on the tumor suppressor microRNA-34. *Cancer Res.* 70, 5923–5930
78 Obad, S. *et al.* (2011) Silencing of microRNA families by seed-targeting tiny LNAs. *Nat. Genet.* 43, 371–378
79 Sethupathy, P. *et al.* (2006) TarBase: a comprehensive database of experimentally supported animal microRNA targets. *RNA* 12, 192–197