

# MicroRNA expression and function in cancer

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**MicroRNAs are small non-coding RNAs of 19–24 nucleotides in length that downregulate gene expression during various crucial cell processes such as apoptosis, differentiation and development. Recent work supports a role for miRNAs in the initiation and progression of human malignancies. Large high-throughput studies in patients revealed that miRNA profiling have the potential to classify tumors with high accuracy and predict outcome. Functional studies, some of which involve animal models, indicate that miRNAs act as tumor suppressors and oncogenes. Here, we summarize miRNA-profiling studies in human malignancies and examine the role of miRNAs in the pathogenesis of cancer. We also discuss the implications of these findings for the diagnosis and treatment of cancer.**

## The new age of cancer: the ‘miR-evolution’

MicroRNAs are small non-coding RNA (see Glossary) of 19–24 nucleotides in length that were discovered >12 years ago by Victor Ambros and colleagues [1]. These authors found that *lin-4*, a known gene involved in the nematode *Caenorhabditis elegans* development, does not code a protein but, instead, gives origin to a small RNA of 22 nucleotides in length that was subsequently shown to interact with the 3' untranslated region (UTR) of the *lin-14* mRNA and to repress its expression [2]. This fascinating form of gene regulation where a small RNA binds to another RNA was largely overlooked for >30 years. MiRNAs have escaped detection, perhaps because of their size: avid gene hunters were mainly interested in long mRNAs and disregarded very short RNAs [3].

Findings over the past five years have strongly supported a role for miRNAs in the regulation of crucial processes such as cell proliferation [4], apoptosis [5], development [6], differentiation [7] and metabolism [8]. Recently, miRNA expression has been linked to cancer. The first evidence that miRNAs are involved in cancer came from the finding that *miR-15a* and *miR-16-1* are downregulated or deleted in most patients with chronic lymphocytic leukemia (CLL) [9]. *Mir-15a* and *miR-16-1* are located within the intron of a non-coding RNA gene of unknown function, called deleted in lymphocytic leukemia 2 (*DLEU2*). This gene resides in a 30-kb region at chromosome 13q14 that is deleted in >65% of CLL cases [9], in 50% of mantle-cell lymphomas [10], in 16–40% of multiple

myeloma [11] and in 60% of prostate cancers [12]. This observation led researchers to investigate the association of miRNA genomic locations and genomic regions that are involved in cancer. Strikingly, 50% of the known miRNAs are located inside or close to fragile sites and in minimal regions of loss of heterozygosity, minimal regions of amplifications and common breakpoints associated with cancer [13]. For example, the cluster *17-92* is located at 13q31, a region commonly amplified in lymphomas [14]; *miR-143* and *miR-145* are located at 5q33, which is frequently deleted in myelodysplastic syndromes, and *miR-142* is located 50 nucleotides from the t(8;17) breakpoint region, which involves chromosome 17 and *MYC*. This translocation juxtaposes the *MYC* gene close to the *miR-142* promoter inducing an abnormal *MYC* overexpression that is associated with lymphomas and polymphocytic leukemia [15] (Table 1).

The use of miRNA microarrays made possible large profiling studies in cancer patients, confirming that miRNAs are differentially expressed in normal and tumor samples [16]. The significance of such deregulation warrants further explanation. Although the function of few cancer-relevant miRNAs has been revealed [16], that of most of them remains to be discovered.

Here, after providing some background on miRNA biogenesis, we summarize miRNA profiling studies in cancer patients and discuss the role of miRNAs in the pathogenesis of human malignancies. The implications for future research and potential therapeutic strategies will also be briefly outlined.

## MicroRNA biogenesis

MiRNAs are initially transcribed by RNA Polymerase II (pol II) into a longer primary transcript (pri-miRNA) of several kilobases in length [17]. These RNAs are capped, polyadenylated and subsequently cleaved in the nucleus by the enzyme Drosha to liberate another precursor of

## Glossary

**Loss of heterozygosity (LOH):** a situation where one chromosome has a normal allele of a gene and one chromosome has a mutant or deleted allele.

**MicroRNome (miRNome):** it is defined as the full complement of miRNAs in the genome.

**Non-coding RNA:** the term non-coding RNA is commonly employed for RNA that does not encode a protein. These non-coding RNA includes ribosomal RNAs (rRNAs) and transferase RNAs (tRNAs), which are involved in mRNA translation, small nuclear RNAs (snRNAs), which are involved in splicing, small nucleolar RNAs (snoRNAs), which are involved in the modification of rRNAs, and miRNAs.

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Available online 30 October 2006.

**Table 1. Examples of microRNAs located in minimal deleted regions, minimal amplified regions and breakpoints involved in human cancers<sup>a,b</sup>**

Chromosome	Location (defining markers)	Size (Mb)	MiR	Hystotype	OG or TS
3p21.1–21.2-D	ARP–DRR1	7	<i>Let-7g</i> and <i>miR-135–1</i>	Lung and breast cancer	Not known
3p21.3(AP20)-D	GOLGA4–VILL	0.75	<i>MiR-26a</i>	Epithelial cancer	Not known
3p23–21.31(MDR2)-D	D3S1768–D3S1767	12.32	<i>MiR-26a</i> and <i>miR-138–1</i>	Nasopharyngeal cancer	Not known
5q32-D	ADRB2–ATX1	2.92	<i>MiR-145</i> and <i>miR-143</i>	Myelodysplastic syndrome	Not known
9q22.3-D	D9S280–D9S1809	1.46	<i>MiR-24–1</i> , <i>miR-27b</i> and <i>miR-23b</i> ; <i>let-7a-1</i> , <i>let-7f-1</i> and <i>let-7d</i>	Urothelial cancer	<i>PTC</i> and <i>FANCC</i>
9q33-D	D9S1826–D9S158	0.4	<i>MiR-123</i>	NSCLC	Not known
11q23–q24-D	D11S927–D11S1347	1.994	<i>MiR-34a-1</i> and <i>miR-34a-2</i>	Breast and lung cancer	<i>PPP2R1B</i>
11q23–q24-D	D11S1345–D11S1328	1.725	<i>MiR-125b-1</i> , <i>let-7a-2</i> and <i>miR-100</i>	Breast, lung, ovary and cervix cancer	Not known
13q14.3-D	D13S272–D13S25	0.54	<i>MiR-15a</i> and <i>miR-16a</i>	B-cell CLL	Not known
13q32–33-A	stSG15303–stSG31624	7.15	<i>MiR-17</i> , <i>miR-18</i> , <i>miR-19a</i> , <i>miR-20</i> , <i>miR-19b-1</i> and <i>miR-92–1</i>	Follicular lymphoma	Not known
17p13.3-D	D17S1866–D17S1574	1.899	<i>MiR-22</i> , <i>miR-132</i> and <i>miR-212</i>	HCC	Not known
17p13.3-D	ENO3–TP53	2.275	<i>MiR-195</i>	Lung cancer	<i>TP53</i>
17q22–t(8;17)	MiR-142s–c-MYC		<i>MiR-142s</i> and <i>miR-142as</i>	Prolymphocytic leukemia	<i>c-MYC</i>
17q23-A	CLTC–PPM1D	0.97	<i>MiR-21</i>	Neuroblastoma	Not known
20q13A	FLI33887–ZNF217	0.55	<i>MiR-297–3</i>	Colon cancer	Not known
21q11.1-D	D21S1911–ANA	2.84	<i>MiR-99a</i> , <i>let-7c</i> and <i>miR-125b</i>	Lung cancer	Not known

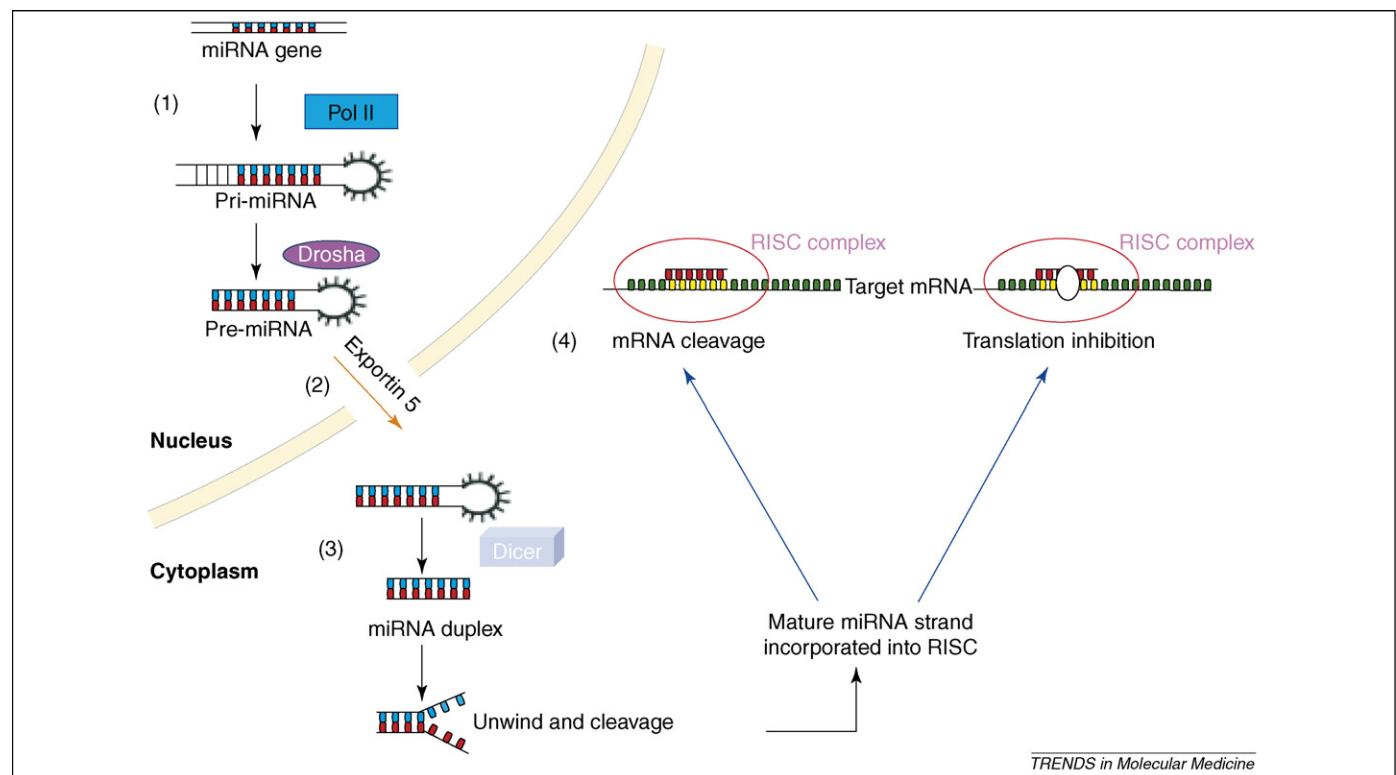
<sup>a</sup>This Table was reproduced, with permission, from Ref. [13].

<sup>b</sup>CLL, chronic lymphocytic leukemia; FANCC, Fanconi anemia, complementation group C; HCC, hepatocellular carcinoma; NSCLC, non-small cell lung carcinoma; OG, oncogene; PPP2R1B, protein phosphatase 2, regulatory subunit A,  $\beta$  isoform; PTC, papillary thyroid carcinoma TP53, tumor protein 53; TS, tumor suppressor.

~60–75 nucleotide (pre-miRNA) [18,19] (Figure 1). Approximately 50% of all miRNAs are embedded within introns of protein-coding genes or non-coding RNA transcripts [20]. This observation suggests that a large number of miRNAs might be transcriptionally linked to the expression of their host-gene promoters. Additionally, some miR-

NAs are clustered in polycistronic transcripts, enabling a coordinated expression.

The miRNA precursor (pre-miRNA) is exported to the cytoplasm by exportin 5 [21,22] and it is further processed by the enzyme Dicer, resulting in a mature product of 19–24 nucleotide duplex, one strand of which is incorporated



**Figure 1.** MicroRNA biogenesis. (1) MiRNAs are transcribed by RNA polymerase II (pol II) into long primary miRNA transcripts of variable size (pri-miRNA), which are recognized and cleaved in the nucleus by the RNase III enzyme Drosha, resulting in an hairpin precursor form called pre-miRNA. (2) This pre-miRNA is exported from the nucleus to the cytoplasm by exportin 5 and is further processed by another RNase enzyme called Dicer (3), which produces a transient 19–24 nucleotide duplex. Only one strand of the miRNA duplex (mature miRNA) is incorporated into a large protein complex called RISC (RNA induced silencing complex). (4) The mature miRNA leads RISC to cleave the mRNA or induce translational repression depending on the degree of complementary sites between the miRNA and its target.

into an effector complex called RNA-induced silencing complex (RISC) [23,24]. The strand that is incorporated into the RISC is the mature miRNA, whereas the opposite strand is eliminated by cleavage or a bypass mechanism [25,26]. MiRNAs regulate their targets by direct cleavage of the mRNA or by inhibition of protein synthesis, according to the degree of complementarities with their targets' UTR regions [27]. Perfect or nearly perfect complementarities between miRNA and its target 3' UTR induce RISC to cleave the target mRNA, whereas imperfect base matching induces mainly translational silencing of the target (Figure 1) but can also reduce the amount of the mRNA target [28]. In the past two years, it has been shown that miRNAs also direct rapid deadenylation of target mRNAs, leading to a rapid mRNA decay and reduction of its levels [29].

Although initially miRNAs were thought to be characteristic of nematodes only, systematic cloning of small RNAs from diverse organisms from plants to humans revealed an increasing number of small RNAs that has been conserved during evolution [30–32]. There are now 462 human miRNAs annotated in the miRNA registry (<http://microrna.sanger.ac.uk/>) [33], and a recent work has predicted the number to grow to 1000 [34]. It is estimated that up to 30% of the human genes are regulated by miRNAs [35]. Each miRNA is supposed to target several hundreds of transcripts [35], making miRNAs one of the biggest family of genome regulators.

### MicroRNA expression profiling in cancer patients

Several groups have studied the miRNA expression in cancer patients and found that miRNAs are differentially expressed in normal and tumor tissues [16]. These differences are tumor-specific and, in some cases, are associated with prognosis [16]. Recent studies on miRNA expression in solid tumors and hematological malignancies are the subject of this section.

#### Expression studies in solid tumors

The first report of miRNA deregulation in solid tumors was the observation of a consistent downregulation of *miR-143* and *miR-145* in colorectal cancer [36].

A reduction of >80% of *let-7a* expression was found by northern blotting in 44% (7 out of 16) of patients with lung cancer compared with healthy controls. More importantly, low levels of *let-7a* were associated with short post-operative survival in 148 lung-cancer patients [37]. Another study profiled 104 pairs of patients with primary lung cancer and healthy controls using a custom microchip containing 368 oligonucleotides in triplicate, which corresponded to 245 precursors and mature miRNAs from human and mouse genomes [38]. A unique miRNA signature consisting of 42 miRNAs could distinguish with accuracy tumor versus normal tissues. Among the genes from the signature, many were associated with fragile sites and regions that are amplified in cancer; in particular, *miR-21* and *miR-205* are located in a region amplified in lung cancer, whereas *miR-126* at 9q34.3 is located in a region commonly deleted in lung cancer. Furthermore, miRNA profiles correlate with lung adenocarcinomas patients' survival: high *miR-155* and low *let-7a-2* expression corre-

lated with poor survival by univariate and multivariate analysis [38]. The miRNA expression profiling associated with survival in lung cancer was confirmed in an independent set of patients using a different technology (real time-PCR) for precursors [38].

In breast cancer, the first microarray profiling study reported results from 76 breast-cancer patients and ten normal breast samples and found 29 miRNAs, the expression of which is significantly deregulated in tumors [39]. A set of 15 miRNAs correctly predicted the nature of the breast-cancer sample analyzed with 100% accuracy. The most-deregulated miRNAs were *miR-125b*, *miR-145*, *miR-21* and *miR-155*. Furthermore, the expression of miRNAs was correlated with specific breast-cancer pathological features such as estrogen-receptor status, tumor stage, vascular invasion and proliferative index [39]. Similar to lung cancer, in breast tumors a reduced expression of *let-7a* was correlated with bad prognosis, characterized by lymph node metastasis and high proliferative index.

In glioblastoma multiforme, the most-frequent and malignant form of primary brain tumors, profiling of cell lines and tumors from patients identified a group of miRNAs, the expression of which is significantly altered in this tumor [40]. Among them, *miR-21*, *miR-221* and *miR-25* are members of a signature that is common to six solid cancers [41].

In a recent work on miRNA expression in papillary thyroid carcinoma (PTC), a set of five miRNAs (which included *miR-221*, *miR-222* and *miR-146*) distinguished unequivocally between PTC and normal thyroid [42]. Tumors with strong upregulation of *miR-221*, *miR-222* and *miR-146* showed a dramatic loss of *KIT* transcript and protein. This study [42] also showed polymorphisms in five out of ten PTCs in the *KIT* 3'UTR that corresponds to the site of interaction with *miR-221*, *miR-222* and *miR-146*, suggesting an altered miRNA–target interaction. Both *miR-221* and *miR-222* have been described to downregulate *KIT* oncogene [43]. The ectopic expression of *miR-221* and *miR-222* inhibits the growth of the erithroleukemic cell line TF-1 in a *KIT*-dependent manner. Collectively, these data support the function of *miR-221* and *miR-222* as oncogenes by downregulation of the *KIT* gene.

Using a similar microarray platform that includes probes to detect precursors and mature miRNAs, Murakami *et al.* [44] reported miRNA expression in 25 pairs of hepatocellular carcinoma and adjacent non-tumoral tissue, and nine chronic-hepatitis tissues. Their analysis showed a small number of miRNAs associated with differentiation state of the tumors, suggesting that these miRNAs might contribute to both oncogenesis and loss of differentiation [44].

Volinia *et al.* [41] performed a large genome-wide miRNome (the full complement of miRNAs in the genome) analysis on 540 samples including breast, lung, stomach, prostate, colon and pancreatic tumors, and identified a solid-cancer signature composed by a large portion of over-expressed miRNAs, including *miR-155*, *miR-17-5p*, *miR-20a*, *miR-21*, *miR-92* and *miR-106a*. The predicted targets for the differentially expressed miRNAs are enriched in genes that encode tumor suppressors and oncogenes ( $p < 0.0001$ ). For example, experimental proof that *miR-106* targets retinoblastoma (*Rb*) gene and that *miR-20*

targets transforming growth factor  $\beta$  receptor II (*TGFBR2*) gene was reported [41].

#### Expression studies in hematological malignancies

Our group has previously reported significant differences in miRNome expression between human CLL B cells and CD5<sup>+</sup> B cells (which represent the normal counterpart to CLL B cells) [45]. At least two distinct clusters were associated with the presence and absence of zeta-chain-associated protein kinase 70 (ZAP-70) expression, a predictor of early disease progression, and with the presence or absence of mutations in the expressed immunoglobulin (Ig) heavy-chain variable region (Vh) genes [45].

Another study from our group has shown for the first time a miRNA signature composed of only 13 genes (out of 190 analyzed) associated with prognosis and progression in CLL [46]. This signature distinguishes cases of CLL with indolent behavior and better prognosis (low levels of ZAP-70 and mutated IgVh) from cases with a more-aggressive course and a shorter interval between diagnosis and the beginning of therapy (high levels of ZAP-70 and non-mutated IgVh).

Interestingly, among the genes associated with prognosis high *miR-155* expression has been correlated with short survival in lung cancer [38]. We also found for the first time [38] germ-line or somatic mutations in five out of 42 sequenced miRNAs in 11 of 75 patients with CLL. However, such mutations were not found in 160 individuals without cancer ( $p < 0.001$ ) [46]. Moreover, in a patient a germ-line point mutation in *miR-16-1* and *miR-15a* primary precursors along with a deletion of the normal allele were responsible for the low expression of the mature miRNAs *in vitro* and *in vivo*.

In other hematological malignancies, high expression of *miR-155* and its host gene B-cell integration cluster (*BIC*) have been reported in pediatric Burkitt lymphoma (BL) [47], Hodgkin disease, and primary mediastinal and diffuse large cell lymphomas [48]. A 100-fold upregulation of the *miR-155* precursor has been reported in pediatric BL, whereas in adult BL lack of *BIC* and *miR-155* was observed [49]. This difference might be related to the age of onset of BL.

Using a novel bead-based hybridization technology, Lu *et al.* [50] measured the expression of 217 human miRNAs in a panel of 334 samples that included multiple human cancers and normal tissues. They observed differential expression of nearly all miRNAs across cancer types. Unsupervised hierarchical clustering revealed that samples grouped according to their developmental origin had clear differences between hematopoietic and epithelial cancers. Stratification within the same tumors was also achieved; for example, in all samples clear partitioning was observed according to defined molecular subtypes such as *BCR-ABL* positive samples. Furthermore, miRNA profiling was more accurate than cDNA arrays to classify a set of 17 poorly differentiated tumors where histological appearance was not diagnostic. Lu *et al.* [50] also observed a general downregulation of miRNAs compared with normal tissues (129 out of 217,  $p < 0.05$ ), suggesting that lower levels of miRNAs reflect a loss of differentiation state typical of cancer. This downregulation of miRNAs in cancer

was not observed in the microarray profiling study of 504 cancer patients [41]. One possible explanation for the discrepancy present in these two large miRNA profiling studies [41,50] might be the different spectrum of analyzed samples: Volinia *et al.* [41] analyzed only solid cancers, whereas Lu *et al.* [50] focused mainly on hematopoietic tumors. However, before definitive conclusions, more independent studies must be performed to reproduce these data.

Overall, these studies have demonstrated the existence of miRNA signatures that are tissue- and tumor-specific and enable to classify, diagnose and, in some cases, predict outcome accurately in cancer patients.

#### MicroRNAs as oncogenes and tumor suppressors

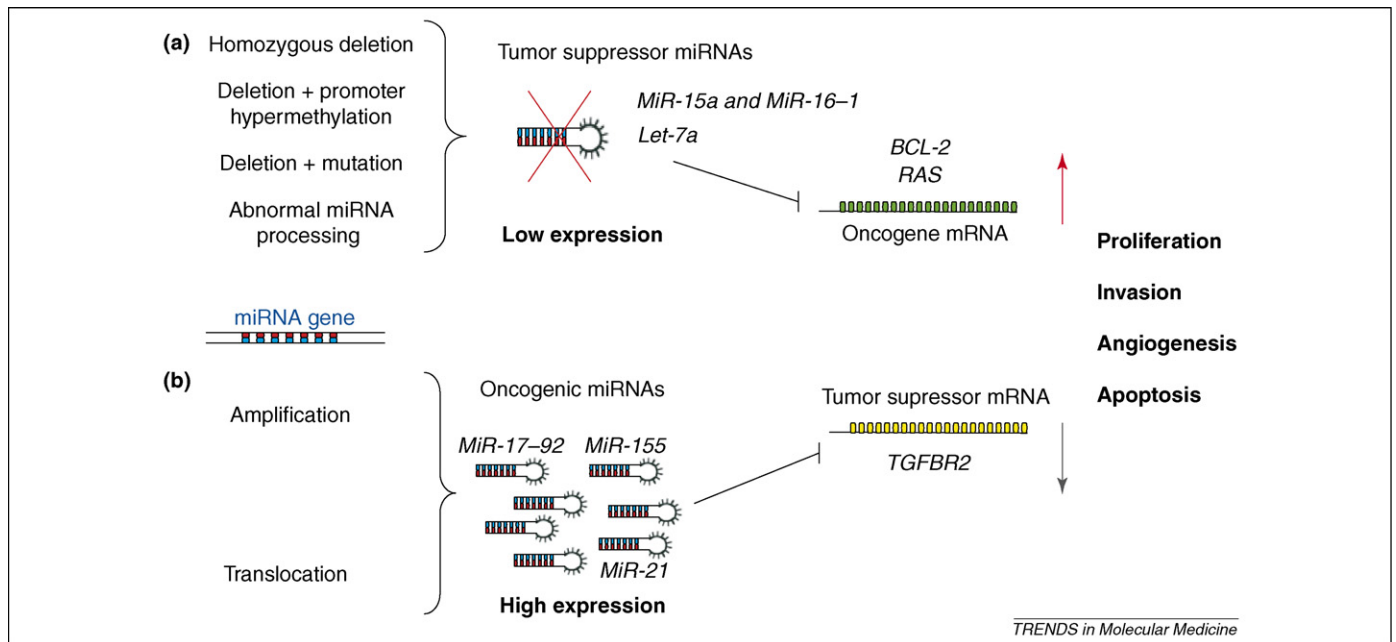
The deregulation of miRNA expression observed in cancer patients needs to be understood. Hypothetically, a miRNA that is downregulated in cancer and targets an oncogene might act as a tumor suppressor, whereas an upregulated miRNA that targets a tumor suppressor or a gene important for differentiation might act as an oncogene [16] (Figure 2). Although expression profiling studies and the association of miRNAs location with fragile sites and genomic areas that are amplified in cancer suggest that miRNAs might function as oncogenes and tumor suppressors [16], definitive evidence linking miRNAs with the development of cancer is scarce (Table 2).

The first evidence that suggests a role of tumor suppressor for miRNAs came from the observation of frequent downregulation of *miR-15a* and *miR-16-1* in CLL patients [9]. These two miRNAs are located in a commonly deleted area in CLL and a study by Cimmino *et al.* [51] showed that *miR-15a* and *miR-16-1* act both as tumor suppressors by downregulating B-cell CLL/lymphoma 2 (*BCL2*), a gene that is frequently upregulated in CLL [52]. Therefore, downregulation of *miR-15a* and *miR-16-1*, which is mainly due to a genetic deletion, might promote B-cell proliferation in CLL, a disease where the main feature is an increased proliferation of mature B cells.

The *let-7* family contains miRNAs that have been shown to regulate the rat sarcoma viral oncogene homolog (*RAS*) family of oncogenes through post-transcriptional repression [53]. Interestingly, *let-7* family members map to fragile sites associated with lung, breast, urothelial and cervical cancer [13]. Profiling studies have shown that low expression of *let-7a* in lung-cancer patients correlates with shortened post-operative survival [37,38]. Furthermore, overexpression of *let-7a* in a human adenocarcinoma cell line inhibits cellular proliferation, indicating that this might be a potential therapeutic approach to treat lung cancer [37].

Two miRNAs located within a fragile site at 5q32–33, *miR-143* and *miR-145*, are downregulated in colon- and breast-cancer patients [36,39,54]. Although many interesting candidate oncogenes are among their predicted targets, no functional studies have been published yet.

Two recent articles by He *et al.* [55] and O'Donnell *et al.* [56] have indicated a clear association between miRNAs and cancer. He *et al.* [55] using a custom array have shown that the *miR-17-92* polycistron, which is located in 13q31–32, a region commonly amplified in B-cell lymphoma, was



**Figure 2.** MicroRNAs as tumor suppressors or oncogenes. **(a)** In this model, we propose that a miRNA that normally downregulates an oncogene can function as a tumor suppressor gene when lost in a tumor. The loss of function of this miRNA by mutations, deletions, promoter methylation or any abnormalities in the miRNA biogenesis might result in an abnormal expression of the target oncogene, which subsequently contributes to tumor formation. Some of the proposed mechanisms for inactivation of miRNAs in cancer are experimentally proven, such as the identification of homozygous and heterozygous deletions at 13q14.3 in B-cell CLL, where *miR-15a* and *miR-16-1* are located [9,46]. In addition, germ-line mutations were found in the precursor of this cluster. **(b)** The amplification or overexpression of a miRNA that downregulates a tumor suppressor or other important genes involved in differentiation might contribute also to tumor formation by stimulating proliferation, angiogenesis and invasion. For example, amplifications of the oncogenic miRNAs, *miR-17-92* cluster, *miR-21* and *miR-155* have been clearly associated with tumor initiation and progression [39,55,56,60].

upregulated in 65% of the B-cell lymphoma samples tested. To test whether this polycistron contributes to cancer formation, the authors used a well-characterized mouse model of B-cell lymphoma induced by the *MYC* gene. Using retroviral transduction, the researchers overexpressed the *miR-17-92* cluster in hematopoietic stem cells from mice that carry the *MYC* transgene. The enforced expression of the *miR-17-92* cluster acted, together with *MYC* expression, to accelerate tumor development [55].

O'Donnell *et al.* [56] has taken the advantage of an inducible system of *MYC* expression and showed that

*MYC* activates the expression of the *miR-17-92* cluster at chromosome 13q31. Chromatin immunoprecipitation experiments showed that *MYC* binds directly to this locus. Furthermore, they indicated that E2F transcription factor 1 (*E2F1*) is downregulated by two miRNAs of the cluster, *miR-17-5p* and *miR-20a*. *E2F1* is a direct target of *MYC* that promotes cell-cycle progression. Thus, *MYC* simultaneously activates *E2F1* and limits its translation through a miRNA-based mechanism, enabling a tightly controlled proliferative signal [56]. These two studies seem contradictory: He *et al.* [55] suggest an oncogenic role for

**Table 2. Experimental data supporting a role for microRNAs in cancer development<sup>a</sup>**

MicroRNA	Genomic location	Expression in patients	Experimental data	Function	Refs
<i>MiR-15a</i> <i>MiR-16-1</i> <i>Let-7a-2</i>	13q14 11q24	Down in CLL and in pituitary adenoma Down in lung cancer	Downmodulate <i>BCL-2</i> Downmodulates <i>RAS</i> and induces apoptosis in lung cancer cell lines	TS TS	[9,51,68] [37,38,53]
<i>MiR-155</i>	21q21	Up in bad prognosis CLL and lung cancer Up in breast cancer, lymphoma Hodgkin and pediatric BL.	Induces pre-B lymphoma and/or leukemia in mice	OG	[38,39,46-48,60]
<i>MiR-17-92</i> cluster <i>MiR-21</i>	13q14 17q23	Up in lymphomas and lung cancer Up in pancreas, glioblastoma and breast cancer	Cooperates with <i>c-MYC</i> ; modulates <i>E2F1</i> Anti-apoptotic in glioblastoma	OG OG	[38,55-57] [39-41,62]
<i>MiR-106a</i>	xq26	Up in lung, gastric and prostate cancer	Downregulates <i>RB-1</i>	OG	[41]
<i>MiR-372</i> <i>MiR-373</i> <i>MiR-142</i>	19q13 17q22	Up in testicular germ-cell tumor cell lines t(8;17) <i>c-MYC</i> is translocated downstream of the <i>miR-142</i> hairpin, resulting in B-lymphoma	Neutralize <i>p53</i> function Enhances <i>MYC</i> expression	OG OG	[61] [15]

<sup>a</sup>BCL-2, B-cell CLL/lymphoma 2; BL, Burkitt lymphoma; CLL, chronic lymphocytic leukaemia; OG: oncogene; RAS, rat sarcoma viral oncogene homolog; RB-1, retinoblastoma 1; TS, tumor suppressor.

*miR-17-92* cluster, whereas O'Donnell *et al.* [56] an anti-apoptotic function (tumor suppressor) for the same cluster. However, it has been reported that high levels of *E2F1* might indeed induce apoptosis [57], so the downregulation of this gene by the *miR-17-92* cluster might function to block the apoptotic activity of *E2F1* and contribute to increase proliferation by *MYC* [54]. The oncogenic role of the *miR-17-92* cluster is further supported by profiling studies showing upregulation of this cluster in B-cell CLL [46] and in solid cancers [48,58]. It is logical to suggest that these miRNAs have distinct roles in distinct cell types, as was formerly proposed for well-known genes that encode tumor suppressors or oncogenes [59].

Another miRNA, *miR-155*, and its host gene, the non-coding *BIC* gene, have also been shown to be consistently upregulated in pediatric BL [47], classical Hodgkin disease, primary mediastinal, diffuse large-cell lymphoma [48], B-cell CLL [46] and, more recently, in lung and breast cancer [38,39]. *MiR-155* is also part of the common signature of six solid cancers [41] and has been linked to early disease progression in CLL [46] and poor survival in lung cancer [38]. Costinean *et al.* [60] have recently shown in a transgenic mouse model that selective overexpression of *miR-155* in B cells induces early B-cell polyclonal proliferation followed by high-grade lymphoma-pre-B leukemia. This is the first evidence that a miRNA by itself can induce a neoplastic disease. However, the mechanism by which *miR-155* induces B-cell proliferation of immature cells is unknown. The fact that this miRNA is also upregulated and associated with prognosis in solid cancers suggests a broader oncogenic function than B-cell differentiation, and might indicate that one (or more) of its targets is (are) important gene(s) linked to a fundamental process that is commonly lost across all cancers irrespective of their origin.

Voorhoeve *et al.* [61] developed a miRNA expression-vector library that contains most cloned human miRNAs and screened for miRNAs that cooperate with oncogenes in cellular transformation [61]. They identified two miRNAs, *miR-372* and *miR-373*, which induced proliferation and tumorigenesis of primary human cells in cooperation with *RAS* by neutralizing wild-type tumor protein p53 (*TP53*) through direct inhibition of the expression of the large tumor suppressor, homolog 2 (*LATS2*). They found that this mechanism participates in the oncogenesis of human testicular germ-cell tumors, enabling oncogenic growth by targeting wild-type *p53* pathway [61]

There is also evidence that *miR-21* functions as an oncogene. This miRNA is upregulated in glioblastoma [40], pancreas [41] and breast cancer [39]. Knock-down of *miR-21* in cultured glioblastoma cells triggers activation of caspases and leads to increased apoptotic cell death [62].

#### Potential therapeutic applications of microRNAs

It has recently been shown that a novel class of chemically engineered oligonucleotides, termed 'antagomirs' effectively silences endogenous miRNAs *in vivo* [63]. An anti-sense oligonucleotide of an abundant liver-specific miRNA, *miR-122*, was constructed using a cholesterol solid support and 2'-OMe phosphoramidites, and injected intravenously in mice. This antisense oligonucleotide efficiently inhibited

the mature *miR-122* in the liver [63]. Using cDNA microarrays, 142 mRNAs were found to be upregulated with at least a 1.4-fold increase in the treated mice [63]. More than 50% of these genes have at least one *miR-122* recognition motifs, corresponding to the mature sequence from nucleotides 2–7, also referred as the 'match seed'. In addition, several pharmacological properties were analyzed in this study [63]. Remarkably, the duration of silencing after a single injection of 240 mg per kilogram of body weight was as long as 23 days, indicating a long-lasting effect. Concerning the bioavailability and silencing activity between different tissues, the antagomir of *miR-16* (a highly expressed miRNAs in all tissues) could silence *miR-16* in all tissues except the brain [63]. Other modified oligonucleotides such as locked nucleic acid (LNA)-modified oligonucleotides can inhibit endogenous miRNAs, leading to upregulation of the cognate target protein [64]. These studies [63,64] showed powerful methods for silencing miRNAs that can be applied to abolish aberrant expression of oncomiRs, such as *miR-155*. However, a word of caution should be raised especially about unwanted effects given the multiplicity of miRNA targets. This will be an important concern in the future when the goal is the restoration of the loss of miRNA expression. Precise and specific delivery to their cell target might overcome this problem.

#### Future directions

Although the discovery of miRNAs will probably change the landscape of cancer genetics, further work is needed to understand the mechanism by which miRNAs contribute to cancer origin and progression. The complexity of miRNA regulation might become even more intricate with: (i) the discovery of other families of non-coding genes with important regulatory functions in normal and disease states [65]; (ii) miRNA–miRNA interactions and regulation of gene promoters by non-coding RNAs, as it has recently been demonstrated [66]. Certainly, bioinformatics will have an important role to unravel the complex network of regulatory genes and their targets. High-throughput target analysis combining cDNA, miRNA and proteomics might help delineating the spectrum of targets that are regulated by miRNAs. More-novel approaches for target identification that use a reverse strategy searching for miRNAs that are attached to the mRNA of a predefined target might also improve the current knowledge [67]. The use of mouse models, conditional transgenic and knock out, for candidate oncomiRNAs will help elucidating the role of miRNA in cancer. The accuracy of miRNAs to classify cancers and its prognostic power remains to be explored in large cohorts of patients. If miRNA profiles can predict outcome and improve stratification, this technology will probably be used in the clinic to help patients. In a similar way, achieving long-lasting and effective silencing of oncomiRNAs might be a great tool to fight cancer.

#### Concluding remarks

New findings over the past few years have catapulted miRNA to the center stage of molecular oncology. Following earlier reports that showed that miRNAs are differentially expressed in cancer, several studies have shown that miRNA profiling is highly accurate to classify tumors and

predict outcome. More importantly, the study of miRNAs provides clues about novel pathways that are disrupted or ectopically activated in cancer and offer potential novel targets for treatment. A bulk of tumor suppressors and oncogenes remains to be discovered and many of them might be non-coding genes. We are scratching the surface of a very complex picture that will probably keep cancer research busy for years to come.

### Acknowledgements

The work in Dr. Croce laboratory is supported by grants from the National Cancer Institute, whereas the CLL Global Research Foundation and the Sidney Kimmel Foundation for Cancer Research support Dr. Calin research. We apologize to our many colleagues whose works were not cited due to space limitations.

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