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Review

miRNAs: Little known mediators of oncogenesis

Andrei L. Gartel [a,*], Eugene S. Kandel [b,**]

[a] *Department of Medicine, University of Illinois at Chicago, 840, South Wood Street, Room 1041, Chicago, IL 60612, United States*
[b] *Department of Cell Stress Biology, BLSC L3-318, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263, United States*

Abstract

Cancer progression is mediated by overexpression of oncogenes and downregulation or loss of tumor suppressors. Proteins, which were traditionally categorized into these groups, have been recently joined by a species of RNA molecules known as microRNAs (miRNAs). miRNAs belong to a class of approximately 22-nt-long non-coding RNAs found in eukaryotes that hinder gene expression by inducing degradation or inhibiting translation of select mRNAs. A growing number of miRNAs have been implicated in promoting or suppressing tumorigenesis in a variety of tissues. The supporting evidence ranges from suggestive expression profiling data to direct functional validation using methods of forward and reverse genetics. We discuss the nature of published results, as well as the merits and pitfalls of various approaches aimed at identification of cancer-related miRNAs and their mRNA targets.

Keywords: RNA interference; MicroRNA; Tumor suppressor; Oncogene

Contents

1. Introduction

Traditional families of protein-coding genes known as tumor suppressors and oncogenes were recently broadened to include short RNA molecules known as microRNAs (miRNAs). These molecules are originally produced by RNA polymerase II as hairpins of longer precursor RNAs and are subsequently processed to approximates 22-nt-long fragments by RNase III enzymes Drosha and Dicer. Mature miRNAs hinder gene expression by promoting mRNA degradation or by inhibiting mRNA translation. The specificity of this process is maintained through short stretches of imperfect homology between miRNA and its targets. At this time, more than 400 human miRNAs have been identified, and each of them may control roughly several hundred target genes to regulate up to 30% of human transcripts [1]. It has been shown that they may act as tumor suppressors by inhibiting oncogenes and being suppressed in cancer, or function as oncogenes by inhibiting tumor suppressors and being overexpressed in tumors. In this article we review the latest functional data that describe the roles and mechanisms of regulation of oncogenic and tumor-suppressor miRNA in cancer.

2. MicroRNAs may act as oncogenes in human cancer

Most of the microRNAs were brought into the spotlight as possible oncogenes because of statistical correlation between their abundance and the cancerous state of various tissues. In general, these data, circumstantial in nature, have to be taken with caution and are insufficient to directly implicate a given molecule as an oncogene. However, this approach could produce a list of candidates suitable for direct individual testing. More-

* Corresponding author. Tel.: +1 312 996 1855.
** Corresponding author. Tel.: +1 716 845 3530.
E-mail addresses: agartel@uic.edu (A.L. Gartel), Eugene.Kandel@RoswellPark.org (E.S. Kandel).

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over, it could be correlated with expression levels of mRNAs and proteins in a hope of identifying probable targets. As the field is relatively new, only a few of potentially oncogenic miRNAs have been subjected to the rigors of functional validation, and for only a handful of oncogenic miRNAs targets are well established (Table 1).

Several miRNAs are overexpressed in multiple types of cancer [2–4]. For example, miR-155 is overexpressed in chronic lymphocytic leukemias (CLL), B-cell lymphomas, Hodgkin's lymphomas, Burkitt lymphomas and in human breast cancer cells suggesting that it may act as oncogene [5–9]. Mir-155 was dramatically overexpressed in CLL patients, but its genomic locus was not amplified [5]. It was shown that clinical isolates of B-cell lymphomas, including diffuse large B-cell lymphoma (DLBCL), contain up to 30-fold higher copy numbers of miR-155 than normal B-cells [6]. Paradoxically, while some other miRNA (miR-103 and miR-107) were overexpressed in pancreatic cancer [10], Mir-155 was downregulated. These results suggest that consequences of miRNA overexpression maybe cell type dependent and based on patterns of mRNA expression that are targeted by miRNAs. It has been proposed that the mRNA of transcription factor PU.1, which is required for late differentiation of B-cells, is a potential target for miR-155 [11]. In addition it was shown that miR-155 is a common target of a broad range of inflammatory signals, including IFN-β suggesting a potential link between inflammation and cancer [12], and has an important role in regulating T helper cell differentiation by regulating cytokine production [13].

The most prominent oncogenic cluster is the miR-17-92 polycistron that is also known as OncomiR-1, because it was the first detected miRNA that was acting as a mammalian oncogene [14]. It is located in intron 3 of the C13orf25 gene and it is amplified in human B-cell lymphomas [15], malignant lymphoma cell lines [16] and in lung cancer [17]. Retroviral insertional mutagenesis also indicated that the mir-17-92 miRNA is an oncogene in T lymphoma, because after infection with the SL3-3 murine leukemia virus, mice developed tumors when the SL3-3 provirus integrated near the gene that encodes the mir-17-92 cistron thus increasing the concentration of the oncogenic miRNA [18]. Overexpression of the miR-17-92 cluster together with the c-myc oncogene-induced tumor progression and inhibition of apoptosis in a mouse B-cell lymphoma model [15]. Similarly, it was shown that introduction of miR-17-92, but not the putative coding region of C13orf25, can enhance growth of lung cancer cell [17]. Inhibition of the members of the miR-17-92 cluster, miR-17-5p and miR-20a with anti-sense oligonucleotides selectively induced apoptosis in lung cancer cells overexpressing miR-17-92, suggesting that a sensitive subset of lung cancers is "addicted" to OncomiR [19]. In addition, it has been shown that c-Myc [20] and E2F3 [21,22] may induce expression of the miR-17-92 polycistron by direct binding to the promoter of this cluster. Two miRNAs of this cluster, miR-17-5p and miR-20a negatively regulated E2F1 activity, suggesting that miR-17-92 may promote cell proliferation by shifting from proapoptotic E2F1 to the proliferative E2F3 [21]. In accordance with the notion that miR-17-92 is an anti-apoptotic oncogene, inhibition of miR-20a by anti-sense ologonucleotides resulted in increased apoptosis after doxorubicin treatment [22]. In addition, miR-17-92 may promote neovascularization by targeting thrombospondin-1 and connective tissue growth factor [23].

Using retroviral mutagenesis and a large-scale mouse T lymphocyte tumor screening, high degree of SL3-3 provirus integration upstream of the miR-106a miRNA cistron was found. In the insertional mutants mir-106a was overexpressed up to 20-fold compared with control tumors [24]. These results and data that miR-106a is overexpressed in K562 and U937 leukemia cell lines [25] suggest the mir-106a cistron may play an important role in T-cell oncogenesis. Since the miR-106a cistron is a homolog of the oncogenic mir-17 cistron [26], it is not surprising that mir-106a also acts as an oncogene. Interestingly, the miR-106-363 and the miR-17-92 clusters have very close homology and their members miR-19 and miR-92 are 100% identical in both clusters [27]. Mir-106a is overexpressed in colon, pancreatic and prostate tumors and may target tumor-suppressor Rb [28]. In addition, it has been shown that long primary miRNA cluster, pri-miR-106-363 overexpressed in 46% of human T-cell leukemias tested and this miRNA cluster targets myosin regulatory light chain-interacting protein and retinoblastoma-binding protein 1-like [27]. These data suggest the importance of miR-106-363 cluster in leukemia.

Another prominent oncogenic miRNA is miR-21. Mir-21 is overexpressed in breast cancer [9], malignant human brain tumors, glioblastomas [29,30], CLL [5] and in cervical cancer [31]. MiR-21 was overexpressed in 21 of the 29 studied cervical tumor samples compared with their normal counterparts [31]. In CNS tumor-derived cell lines, levels of miR-21 increased 17.7-fold [30]. In addition, overexpression of miR-21 in pancreatic tumors correlated with liver metastasis [10]. One of the predicted targets of mir-21 is SPOCK1, a proteoglycan that is expressed in neurons [30,32]. Another target is the potential tumor-suppressor gene tropomyosin 1 (TRP1) [33]. It has been shown that TPM1 expression increased in tumors with anti-miR-21 and miR-21 binds to the 3′-UTR of TPM1 mRNA [33]. MiR-21 was highly over-expressed in malignant cholangiocytes and it directly modulated PTEN expression in these cells [34]. Transfection of MCF-7 breast cancer cells with anti-miR-21 oligonucleotides led to suppression of both cell growth in vitro and tumor growth in vivo, and to increased programmed cell death [35]. Similarly, knockdown of miR-21 led to induction of apoptosis in cultured glioblastoma cells [29], but, paradoxically, to increased cell growth in HeLa cells [36]. One might attribute this discrepancy to the differences in the expression pattern of individual miR-21 targets or the roles these targets are playing in different cells, although some methodological differences between the contradictory reports could not be ruled out.

MiR-221 is encoded on the X chromosome and is overexpressed in thyroid cancer [3], glioblastoma [37], pancreatic cancer [38] and in prostate cancer [39]. MiR-221 was also overexpressed in unaffected thyroid tissue in some papillary thyroid carcinoma (PTC) patients, suggesting that its upregulation is an early event in tumorigenesis [3]. Ectopic overexpression of miR-221/222 strongly affects growth potential of slowly growing prostate carcinoma LNCaP cells, inhibiting p27 and

inducing increase of their colony forming potential in soft agar. Conversely, miR-221 and miR-222 knockdown by anti-sense oligonucleotides increases levels of p27 in aggressive prostate carcinoma PC3 cells, and strongly reduces their clonogenicity in vitro [39]. Galardi et al. suggest that oncogenic miR-221 targets CDK inhibitor p27, which is dosage-dependent tumor suppressor in prostate cancer [39]. Inhibition of miR-221 expression by small molecules may be new strategy against prostate cancer.

Two microRNAs, miR-372 and miR-373 were identified in a screen for oncogenic miRNAs that can overcome oncogene-mediated cell cycle arrest in cells with wild-type p53. MiR-372&3 cooperated with activated RASV12 and stimulated advanced neoplastic transformation in the presence of active p53, suggesting that these microRNAs may relieve oncogene-induced growth arrest, which is typically invoked by the p53–p21 pathway [40]. These miRNAs target LArge Tumor-Suppressor homolog 2 (LATS2), a serine-threonine kinase that inhibits cyclin E/CDK2 activity and RASV12-mediated transformation [41]. Testicular germ cell tumors (TGCTs) of adolescents and adults often carry wildtype p53 and are sensitive to chemotherapies and irradiation [42], suggesting a mutation that undermines the function, but not the structure of p53. Experiments showed that miR-372&3 cluster expressed in the majority of primary TGCTs, and their expression correlated with wildtype status of p53 [40]. In addition, cotransfection experiments showed that miR-372 and miR-373, similarly to Cyclin E/CDK2, were able to overcome a p21-mediated cell cycle arrest in TGCTs [40]. These data suggest that oncogenic miR-372&3 are involved in the formation of testicular germ cell tumors by inhibiting of p53/p21 activity and allowing growth of tumors in the presence of wild-type p53.

Let-7 miRNA is a prominent tumor suppressor (see Section 3) that targets RAS [15], but RAS mRNA levels are only weakly affected by let-7a-3, the member of let-7 family. The human let-7a-3 gene is embedded in a well-defined CpG island and is heavily methylated by DNA methyltransferases DNMT1 and DNMT3B in normal human tissues. Paradoxically, hypomethylation of this gene in lung cancer correlates with advanced tumor phenotype [43], prompting speculation that of let-7a-3 targets may be tumor suppressors, and that abnormal methylation/demethylation of the miRNA genes might contribute to the development of human cancer.

A large-scale miRnome analysis on 363 solid primary tumors and 177 normal tissues samples confirmed overexpression of major oncogenic miRNA in cancer, including miR-17-5p, miR-20a, miR-21, miR-92, miR-106a and miR-155 [28]. These results suggested that oncogenic miRNAs are broadly implicated in cancer pathogenesis of solid tumors. To determine miRNA DNA copy number abnormalities in cancer, Zhang et al. analyzed 283 human miRNA genes by comparative genomic hybridization in 227 human samples of melanoma, ovarian and breast cancer [4]. They found high DNA copy number alterations in ovarian cancer (37.1%), breast cancer (72.8%) and melanoma (85.9%) and they identified 26 miRNA genes with gene copy number gains and high level of expression that were shared among all three studied cancer types [4]. These results suggest that copy number alterations of miRNAs are common in cancer and may explain frequent miRNA gene upregulation observed in a variety of tumors.

Moreover, it was recognized that patterns of miRNA expression in cancer could be used for classification of human tumors. For example, cancers from comparable developmental origins were accurately grouped together, whereas data from mRNA microarrays did not reveal accurate classification [44]. The analysis of 217 mammalian miRNAs from a panel of more than 200 human cancers showed general downregulation of miRNAs expression in tumors compared to normal tissues, suggesting that the majority of miRNAs may be tumor suppressors [44]. However, these results were not confirmed by Volinia et al. [28], either because of the difference in number of samples or due

Table 1
Examples of microRNAs with predicted oncogenic properties

miRNA	Alteration in cancers	Effect of overexpression	Proposed targets	Reference
Mir-17-92, MiR-17-5p and miR-20a	Overexpressed in B-cell lymphoma and lung cancer	Tumor progression, inhibition of apoptosis and promotion of angiogenesis	E2F1, thrombospondin-1 and connective tissue growth factor	[15–17,19,21–23]
Mir-106a, MiR-106 and -363	Overexpressed in T-cell leukemia, colon, pancreatic and prostate tumors		Retinoblastoma protein, myosin regulatory light chain-interacting protein and retinoblastoma-binding protein 1-like	[24,25,27,28]
MiR-155	Overexpressed in CLL, B-cell, Hodgkin's and Burkitt lymphomas and in human breast cancer		PU.1, human angiotensin II type 1 receptor	[5–9,11,76]
MiR-21	Overexpressed in breast cancer, glioblastomas, CLL and in cervical cancer	Liver metastasis, induction of tumor cells growth in vitro and in vivo, inhibition of apoptosis and inhibition of cell growth	SPOCK1, tropomyosin 1 and PTEN	[5,9,10,29–31,33–35]
MiR-221	Overexpressed in thyroid cancer, glioblastoma, pancreatic cancer and in prostate cancer	Increase growth and colony forming potential of slowly growing prostate carcinoma cells	p27	[3,37–39]
MiR-372, miR-373	Overexpressed in testicular germ cell tumors with wt p53	Overcome p21-mediated cell cycle arrest	LATS2	[40]

to the different technical platforms in these studies. It is also possible that a strong selective pressure to lose expression of several tumor-suppressor miRNAs in cancerous cells results in the general alteration of miRNA production, while most of the lost miRNAs are not directly relevant to oncogenesis. In any case, it is obvious that in addition to the oncogenes, miRNAs include a sizeable number of tumor suppressors.

3. MicroRNAs may act as tumor suppressors

Traditionally, a tumor suppressor is functionally defined as a gene whose inactivation results in cancer. This deceptively simple definition is hard to satisfy. Ideally, this is done by showing that (i) the gene is found altered in tumor samples or is linked to a heritable disease and (ii) its specific inactivation in model systems causes tumor formation or progression. However, taken individually, these phenomena may denote, for example, a marker mutation for a tumor-prone haplotype, or an artificially created defect that is irrelevant to human disease. Even more circumstantial are the evidences that overexpression of the gene leads to growth arrest or cell death: while a tumor suppressor may indeed act this way, so are a multitude of other factors whose overexpression is deleterious. It is not surprising that the path from initial identification to full confirmation takes many years. The actual mechanism of tumor suppression is even more elusive: even canonical tumor suppressors scrutinized by decades of research may present new and unexpected features.

Assignment of tumor-suppressor function and a relevant target to a miRNA faces some peculiar technical difficulties that are akin to the ones occurring in the experiments with experimental RNA interference. These issues and the essential controls were discussed in detail elsewhere [45]. For example, such a miRNA is expected to reduce growth or survival of cells, but toxicity is also the most common manifestation of non-specific effects. Similarly, overexpression of its target is expected to overcome the growth inhibition or toxicity, yet the putatively relevant targets are growth enhancing or anti-apoptotic. Consequently, their overexpression may have the expected effect even if the primary target of the miRNA is different.

In the view of these many challenges it is not surprising that characterization of tumor suppressors among mammalian microRNAs, which came into the spotlight only several years ago, is far from complete. What has been achieved, however, is quite exciting (Table 2).

A vast body of evidence indicates that some miRNAs are frequently reduced in malignant cells, while the corresponding genes are involved in deletions and rearrangements. Seminal work from the group of Dr. Croce has shown that miR15a and miR16-1 are the targets of frequent rearrangements and occasional mutations in chronic lymphocytic leukemia [46]. Furthermore, the synteneic region in mouse genome has been implicated in CLL predisposition of NZB mice, which carry a point mutation in miR16-1 [47]. Both miR15a and miR16-1 were able to repress Bcl-2 expression and cause apoptosis upon overexpression [48]. It is not clear, however, whether downregulation of Bcl-2 is the only or even the main contribution of these miRNAs to tumor suppression and cell death. One may try to answer this question by attempting to protect the cells from miR15a or miR16-1-induced apoptosis by expression of a Bcl-2 transgene devoid of the miRNA target motifs. The situation is complicated by the fact that Bcl-2 is a very broad suppressor of cell death and its overexpression may protect the cells from the impacts that are primarily aimed at other anti-apoptotic proteins. Of note, the Croce group, as well as Bottoni et al. reported that expression of arginyl-tRNA synthetase (RARS), a candidate target of miR16-1 and an inhibitor of secretion of a tumor-suppressive cytokine EMAP II, was inversely correlated with that of the two miRNAs [46,49]. The significance of the RARS connection for the effects of miR16-1 either in vivo or in vitro is still unclear.

In a similar study, the Croce group reported an inverse correlation between the expression of another anti-apoptotic protein, Tcl1 and two miRNA families, miR29 and miR181 in B-cell [50]. Furthermore, expression of miR29b and miR181b sufficed to suppress a heterologous reporter supplemented with parts of the TclI sequence. Unlike Mir16-1 and miR 15a, miR29 and miR181 have not been associated with common genomic rearrangements. It is still uncertain whether restoration of the physiological levels of these miRNAs would be sufficient to suppress the leukemia growth and whether the loss of one or more of these miRNAs would have an oncogenic effect on B-cells. Interestingly, another pro-survival factor and potential oncogene, Mcl-1, has been reported to be under miR-29 control [51].

P53 is, arguably, the most broadly studied tumor suppressor with pleiotropic functions. It acts as a transcription factor and micrRNAs are found among its targets and may account for some of its activities [52]. In particular, miR-34 family molecules are growth-suppressive in a variety of cellular backgrounds. It remains to be seen whether under any conditions miR-34 are indeed critical for p53-mediated tumor suppression.

MicroRNAs reveal a high degree of evolutionary conservation. Johnson et al. used this observation to extrapolate the connection between C. elegans Ras and let-7 miRNA to mammalian system (see also Section 2) [15]. Remarkably, the genes for at least three Ras family members (HRAS, NRAS and KRAS) retained the binding sequences for the human let-7 family members, and forced changes in the levels of let-7 in human cells were accompanied by reciprocal changes in Ras expression. This let-7–Ras connection may explain growth suppressive effect of ectopically overexpressed let-7 [53,54] and a worse prognosis for lung cancers with let-7 reduction [53]. However, certain caution has to be exercised in interpreting these observations, as contributions of other potential targets of let-7 to these phenomena have not been ruled out. Unfortunately, the critical control, reversion of let-7-mediated growth suppression upon ectopic expression of Ras sans the 3′-UTR, was missing from these experiments. Of note, another oncogene, high mobility group A2 protein (HMGA2) is also a target of let-7 [55,56]. Importantly, the growth-suppressive effect of let-7 in lung cancer cells could be alleviated by overexpression of HMGA2 devoid of let-7 target motifs [55].

DNA methylation is an important factor in gene regulation and is known to contribute to inactivation of tumor suppressors

Table 2
Examples of microRNAs with predicted tumor-suppressive properties

miRNA	Alteration in cancers	Effect of re-expression or overexpression	Proposed targets	Reference
Let-7	Reduced in lung and colon cancers	Growth suppression	H-Ras, N-Ras, K-Ras, HMGA2	[15,53–56]
Mir15a	Lost in CCL, pituitary adenoma	Apoptosis	Bcl-2	[46,48,49]
Mir16-1	Lost in CCL, pituitary adenoma	Apoptosis	Bcl-2; arginyl-tRNA synthetase	[46,48,49]
miR127	Reduced in various cancer cell lines		Bcl-6	[57]
miR29	Reduced in CCL	Sensitization to apoptosis	Tcl1, Mcl1	[50,51]
miR181	Reduced in CCL		Tcl1	[50]
miR124a	Reduced by methylation in colon and lung cancers	Tolerated in a colon cancer cell line	CDK6	[58]
miR17-5p	Decreased in some studies, increased in others	Growth suppression upon individual expression, growth enhancement upon expression of the polycistronic precursor	E2F, AIB1	[4,17,20,22,28,44,60–62]

in human malignancies. In an intriguing study, a large set of miRNAs was reported to be induced by DNA demethylation [57]. Of those, miR-127 was found consistently downregulated in cancer samples. Interestingly, it has been proposed that miR-127 may have more than one promoter and may be expressed either as a part of a large cluster or individually, the latter component being a subject to epigenetic regulation [57]. This miRNA was proposed to be an inhibitor of the Bcl-6 protooncogene and, therefore, its loss may have oncogenic consequences. A functional confirmation of the latter prediction would be a highly significant funding.

In a similar study, Lujambio et al. have reported that expression of a number of miRNAs was elevated when the process of DNA methylation was suppressed and at least some of the corresponding genes contained methylation-prone CpG islands [58]. Furthermore, one such miRNA, miR-124a, was shown to target CDK6. The latter was in agreement with inverse correlation between miR-124a and CDK6 activity in a panel of cancer samples [58], suggesting that the miRNA inactivation bears a functional significance to the disease. If miR-124 is a tumor suppressor, one may predict that its inactivation would be growth-promoting, while its restoration to the normal levels would be growth suppressing. The latter does not appear to be the case in colon cancer HCT116 cells, which are able to proliferate despite the loss of DNA methyltransferase and restored expression of miR-124a. However, there is, at least, a formal possibility that this reflects additional compensatory events, which occurred during the initial establishment of the methyltransferase-deficient derivative of the cell line.

For some microRNAs, the scarcity of functional data does not warrant a direct implication in tumor suppression, despite a well-established negative correlation with the cancerous state of the respective tissue. For example, miR-128, miR-181a, miR-181b and miR-181c are consistently downregulated in glioblastomas, but this change may be a marker of a de-differentiated cancerous cell, rather than a causative event in the disease [37]. The same considerations apply to the findings of miR-122 loss in hepatocellular carcinomas [59].

As more data on miRNA function emerge, the roles of certain miRNAs become controversial. For example, Hossain et al. reported that miR17-5p is an inhibitor of AIB1 oncogene and, therefore, may have a tumor-suppressive function [60]. Accordingly, overexpression of this miRNA suppressed estrogen- and anchorage-independent growth of breast cancer cells. This claim is supported by reported loss of the corresponding locus in a variety of cancers [4]. The issue is complicated by the fact that both AIB1 and miR17-5p are both previously reported regulators of E2F1, which is intimately involved in cell proliferation [20,61]. Furthermore, as mentioned above, miR17-5p is encoded by a polycistronic locus C13orf25 as miR-17-92 cluster ("Oncomir-1"), which is amplified in human cancers and upon overexpresson acts as an oncogene (see Section 2) [17,62]. Two independent studies reported opposite changes in miR17-5p expression in cancer [4,44]. Yet another level of complexity is added by the dependence of transcription of C13orf25 on E2F [22]. In an attempt to resolve the controversy, Hayashita et al. point to the observation that miR17-5p, in fact, has not been reported overexpressed together with other members of the cluster [17], but the latter may be a merely technical issue: detection of miR17-5p is complicated by the presence of several highly homologous miRNAs.

It is interesting to note that a similar ambivalence about tumor promoting and growth suppressive function emerged about most, if not all, alleged oncogenes, including E2F and Myc. For example, in addition to growth-promoting oncogenic signals, Myc is known to initiate apoptotic tumor-suppressive response (reviewed in [63]), while E2F-1 may induce p21 and an S-phase arrest [64]. In fact, most signaling cascades in mammalian cells invoke some negative feedback mechanisms. Conceptually, we may consider the whole miR17-92 cluster, which is normally produced as a single transcript, as one functional unit akin to a protein with multiple domains, with some of its elements contributing to negative feedback. One may predict that in cases when such elements are mutated or lack a downstream effector, the feedback falters, and the net result is unchecked growth enhancement. In the Myc analogy, this would be equivalent to a Myc mutation that reduces its apoptotic, but not growth-enhancing effect, or to a deletion of p53, which makes Myc proapoptotic signaling inefficient (e.g. [65,66]). The analogy between a multi-cistronic cluster of miRNA and a multi-domain protein may be broadly applicable to the analysis of other miRNAs as well.

In addition to the mechanisms that account for lower abundance of miRNA at transcriptional level, there are observations suggesting that processing of miRNA precursors may be subject to regulation as well [67]. The reported massive failure of miRNA processing in cancer might hint at a large-scale involvement of miRNAs in tumor suppression. This finding also underscores the complexicty of miRNA regulation, as well as emphasizes the need to discriminate between processed and unprocessed miRNA forms in expression profiling studies. A better understanding of this phenomenon would not only shed the light on altered miRNA function in cancer, but would also enable better design of miRNA and siRNA expression constructs for experimental and therapeutic RNA interference.

4. Some cancer-related miRNAs are yet to be discovered

While emerging research implicates a large number of miRNAs as modulators of cancerous phenotype, the nascent state of this field makes it virtually certain that many more have yet escaped identification. How do we identify a miRNA and implicate it as a modulator of carcinogenesis? In this review we will focus on the way of establishing the initial link to cancer, as opposed to general techniques of discovering and cloning short RNAs.

One theoretical possibility is a bioinformatics-assisted search, which would rely on predicted targeting of oncogenes or tumor suppressors as one of the criteria for selection of candidates. Such a search could be conducted on the sequences of miRNAs, which were already discovered by other means, or on a full-genome scale, which would include a step of identifying sequences which are likely to encode miRNAs. The number of target-prediction programs described in literature approaches 20, and their detailed comparison is beyond the scope of this review. A subset of these programs and the underlying algorithms are discussed in relatively recent publications [68,69]. In general, the homology between a miRNA and its target may be rather short and imperfect, making a pure in silico discovery uncertain. Perhaps a combination of short stretches of perfect homology at the 5′-end of the miRNA, evolutionary preservation and multiple occurrence of predicted targets within the same mRNA could collectively point to a meaningful candidate miRNA–mRNA pair, yet it is likely to miss some important connections as well [70]. Comparison of miRNA and mRNA expression profiles is used to escape the constraints of interspecies analysis [71]. Megraw et al. compared the output of several programs and concluded that “no one program can be considered as consistently superior to the rest; however, in many cases it may be helpful to use the intersection of the predictions of a subset of these programs” [69]. An obvious complication for the identification of cancer-related miRNA–mRNA interactions is that not all cancer-related mRNAs are known. Consequently, the significance of a properly predicted pair may be easily missed. The situation is similar to the one with transcription factor binding sites, for which many predictions could be made, but experimental validation is still an accepted necessity.

Another approach to identifying cancer-related miRNAs is via some means of comparative expression analysis, such as micro-array hybridization. Comparing the expression profiles between distinct sets of healthy and cancerous cells has led to successful identification of miRNA signatures of specific cancer types. Expression changes per se do not validate a functional involvement in the disease. Earlier, we have presented a few studies that used expression changes as a starting point and validated the candidates by additional experimentation. It is likely that similar cancerous phenotypes could be evoked by multiple alternative mutations; therefore, it is likely that the most ubiquitously deregulated miRNAs represent the markers, rather than the causes of the disease. Examples of this were discussed above. The value of such findings is not limited to diagnostics: ubiquitous changes, albeit not causative, may point to peculiarities of cancerous cells that may be exploited in therapeutic purposes.

An obvious drawback of the hybridization-based approach is that it is limited to the miRNAs that have been discovered previously. In the case of traditional mRNAs, several techniques permit direct identification of differentially expressed molecules. Various forms of differential display or subtractive hybridization use biochemical tricks to select and clone the molecules of interest, while large-scale SAGE profiling can uncover the differentially expressed tags even if their mRNAs of origin have not been previously recognized. Indeed, construction and comparison of large-scale libraries has been used to uncover differentially expressed miRNAs [72].

MicroRNAs with cancer-related roles could be found in functional screens. For example, one may rely on a collection of known miRNAs delivered in a form of an expression library in order to identify the ones with interesting features [40]. Since miRNAs are sensitive to interference from anti-sense molecules, one may screen a collection of such inhibitors in order to select miRNAs whose inhibition creates the phenotype of interest. By conducting such a screen on a relatively small collection of synthetic oligonucleotides, Cheng et al. identified a number of candidate miRNAs that may modulate cell growth and survival [36]. Although the latter work was performed using transfection of synthetic oligonucleotides, it is known that the function of at least some non-coding RNAs could be counteracted by expressing respective anti-sense molecules (e.g. [73]). Therefore, cloning of anti-sense inhibitors of miRNAs should be attainable from expression libraries as well. Of note, de novo cloning of short RNAs could be done directly into an expression vector. However, we are unaware of such attempts and the reported miRNA screens were conducted on already known miRNAs.

Yet another potential method to uncover the miRNAs with a particular function is via insertional mutagenesis. Mutagenesis via random insertion of promoter has emerged as a method of choice for many forward genetics experiments (discussed in Ref. [74]). The procedure may generate both gain-of-function and loss-of-function events and does not discriminate between protein-coding and non-coding targets. In fact, the strength of the approach is emphasized by the fact that the loci encoding microRNA precursors have been identified as cancer-relevant insertion sites even before RNA interference has become commonly known in mammalian cells [75]. With the advent of transposonal vectors, which could be used as cell-type specific

insertional mutagens in vivo, we anticipate that cancer-related miRNAs will be identified in tissue-specific genetic screens conducted in whole animal models. This may open a possibility to identify novel miRNAs that control angiogenesis, immune evasion and other oncogenic phenotypes that are not readily recapitulated in vitro. The need to identify such novel miRNAs, as well as to assign the functional roles to the already known ones, is sure to propel a broad research effort for years to come.

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References

[1] Berezikov E, Guryev V, van de Belt J, Wienholds E, Plasterk RH, Cuppen E. Cell 2005;120(1):21–4.
[2] Jiang J, Lee EJ, Gusev Y, Schmittgen TD. Nucleic Acids Res 2005;33(17):5394–403.
[3] He H, Jazdzewski K, Li W, Liyanarachchi S, Nagy R, Volinia S, et al. Proc Natl Acad Sci U S A 2005;102(52):19075–80.
[4] Zhang L, Huang J, Yang N, Greshock J, Megraw MS, Giannakakis A, et al. Proc Natl Acad Sci 2006;103(24):9136–41.
[5] Fulci V, Chiaretti S, Goldoni M, Azzalin G, Carucci N, Tavolaro S, et al. Blood 2007;109(11):4944–51.
[6] Eis PS, Tam W, Sun L, Chadburn A, Li Z, Gomez MF, et al. Proc Natl Acad Sci U S A 2005;102(10):3627–32.
[7] Kluiver J, Poppema S, de Jong D, Blokzijl T, Harms G, Jacobs S, et al. J Pathol 2005;207(2):243–9.
[8] Metzler M, Wilda M, Busch K, Viehmann S, Borkhardt A. Genes Chromosomes Cancer 2004;39(2):167–9.
[9] Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, et al. Cancer Res 2005;65(16):7065–70.
[10] Roldo C, Missiaglia E, Hagan JP, Falconi M, Capelli P, Bersani S, et al. J Clin Oncol 2006;24(29):4677–84.
[11] John B, Enright AJ, Aravin A, Tuschl T, Sander C, Marks DS. PLoS Biol 2004;2(11):e363.
[12] O'Connell RM, Taganov KD, Boldin MP, Cheng G, Baltimore D. Proc Natl Acad Sci U S A 2007;104(5):1604–9.
[13] Thai TH, Calado DP, Casola S, Ansel KM, Xiao C, Xue Y, et al. Science 2007;316(5824):604–8.
[14] Hammond SM. Curr Opin Genet Dev 2006;16(1):4–9.
[15] Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, et al. Cell 2005;120(5):635–47.
[16] Tagawa H, Seto M. Leukemia 2005;19(11):2013–6.
[17] Hayashita Y, Osada H, Tatematsu Y, Yamada H, Yanagisawa K, Tomida S, et al. Cancer Res 2005;65(21):9628–32.
[18] Wang CL, Wang BB, Bartha G, Li L, Channa N, Klinger M, et al. Proc Natl Acad Sci U S A 2006;103(49):18680–4.
[19] Matsubara H, Takeuchi T, Nishikawa E, Yanagisawa K, Hayashita Y, Ebi H, et al. Oncogene 2007.
[20] O'Donnell KA, Wentzel EA, Zeller KI, Dang CV, Mendell JT. Nature 2005;435(7043):839–43.
[21] Woods K, Thomson JM, Hammond SM. J Biol Chem 2007;282(4):2130–4.
[22] Sylvestre Y, De Guire V, Querido E, Mukhopadhyay UK, Bourdeau V, Major F, et al. J Biol Chem 2007;282(4):2135–43.
[23] Dews M, Homayouni A, Yu D, Murphy D, Sevignani C, Wentzel E, et al. Nat Genet 2006;38(9):1060–5.
[24] Lum AM, Wang BB, Li L, Channa N, Bartha G, Wabl M. Retrovirology 2007;4:5.
[25] Yu J, Wang F, Yang GH, Wang FL, Ma YN, Du ZW, et al. Biochem Biophys Res Commun 2006;349(1):59–68.
[26] Tanzer A, Stadler PF. J Mol Biol 2004;339(2):327–35.
[27] Landais S, Landry S, Legault P, Rassart E. Cancer Res 2007; 67(12):5699–707.
[28] Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, et al. Proc Natl Acad Sci 2006;103(7):2257–61.
[29] Chan JA, Krichevsky AM, Kosik KS. Cancer Res 2005;65(14):6029–33.
[30] Gaur A, Jewell DA, Liang Y, Ridzon D, Moore JH, Chen C, et al. Cancer Res 2007;67(6):2456–68.
[31] Lui WO, Pourmand N, Patterson BK, Fire A. Cancer Res 2007; 67(13):6031–43.
[32] Cifuentes-Diaz C, Alliel PM, Charbonnier F, de la Porte S, Molgo J, Goudou D, et al. Mech Dev 2000;94(1-2):277–82.
[33] Zhu S, Si ML, Wu H, Mo YY. J Biol Chem 2007.
[34] Meng F, Henson R, Lang M, Wehbe H, Maheshwari S, Mendell JT, et al. Gastroenterology 2006;130(7):2113–29.
[35] Si ML, Zhu S, Wu H, Lu Z, Wu F, Mo YY. Oncogene 2007; 26(19):2799–803.
[36] Cheng AM, Byrom MW, Shelton J, Ford LP. Nucleic Acids Res 2005;33(4):1290–7.
[37] Ciafre SA, Galardi S, Mangiola A, Ferracin M, Liu CG, Sabatino G, et al. Biochem Biophys Res Commun 2005;334(4):1351–8.
[38] Lee EJ, Gusev Y, Jiang J, Nuovo GJ, Lerner MR, Frankel WL, et al. Int J Cancer 2007;120(5):1046–54.
[39] Galardi S, Mercatelli N, Giorda E, Massalini S, Frajese GV, Ciafre SA, et al. J Biol Chem 2007.
[40] Voorhoeve PM, le Sage C, Schrier M, Gillis AJ, Stoop H, Nagel R, et al. Cell 2006;124(6):1169–81.
[41] Li Y, Pei J, Xia H, Ke H, Wang H, Tao W. Oncogene 2003;22(28):4398–405.
[42] Mayer F, Stoop H, Scheffer GL, Scheper R, Oosterhuis JW, Looijenga LH, et al. Clin Cancer Res 2003;9(2):767–73.
[43] Brueckner B, Stresemann C, Kuner R, Mund C, Musch T, Meister M, et al. Cancer Res 2007;67(4):1419–23.
[44] Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, et al. Nature 2005;435(7043):834–8.
[45] Gartel AL, Kandel ES. Biomol Eng 2006;23(1):17–34.
[46] Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, et al. Proc Natl Acad Sci U S A 2002;99(24):15524–9.
[47] Raveche ES, Salerno E, Scaglione BJ, Manohar V, Abbasi F, Lin YC, et al. Blood 2007.
[48] Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, et al. Proc Natl Acad Sci U S A 2005;102(39):13944–9.
[49] Bottoni A, Piccin D, Tagliati F, Luchin A, Zatelli MC, degli Uberti EC. J Cell Physiol 2005;204(1):280–5.
[50] Pekarsky Y, Santanam U, Cimmino A, Palamarchuk A, Efanov A, Maximov V, et al. Cancer Res 2006;66(24):11590–3.
[51] Mott JL, Kobayashi S, Bronk SF, Gores GJ. Oncogene 2007.
[52] He L, He X, Lim LP, de Stanchina E, Xuan Z, Liang Y, et al. Nature 2007;447(7148):1130–4.
[53] Takamizawa J, Konishi H, Yanagisawa K, Tomida S, Osada H, Endoh H, et al. Cancer Res 2004;64(11):3753–6.
[54] Akao Y, Nakagawa Y, Naoe T. Biol Pharm Bull 2006;29(5):903–6.
[55] Lee YS, Dutta A. Genes Dev 2007;21(9):1025–30.
[56] Mayr C, Hemann MT, Bartel DP. Science 2007;315(5818):1576–9.
[57] Saito Y, Liang G, Egger G, Friedman JM, Chuang JC, Coetzee GA, et al. Cancer Cell 2006;9(6):435–43.
[58] Lujambio A, Ropero S, Ballestar E, Fraga MF, Cerrato C, Setien F, et al. Cancer Res 2007;67(4):1424–9.
[59] Kutay H, Bai S, Datta J, Motiwala T, Pogribny I, Frankel W, et al. J Cell Biochem 2006;99(3):671–8.
[60] Hossain A, Kuo MT, Saunders GF. Mol Cell Biol 2006;26(21):8191–201.
[61] Oh A, List HJ, Reiter R, Mani A, Zhang Y, Gehan E, et al. Cancer Res 2004;64(22):8299–308.
[62] He L, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S, et al. Nature 2005;435(7043):828–33.
[63] Evan GI, Christophorou M, Lawlor EA, Ringshausen I, Prescott J, Dansen T, et al. Cold Spring Harb Symp Quant Biol 2005;70:263–73.

[64] Radhakrishnan SK, Feliciano CS, Najmabadi F, Haegebarth A, Kandel ES, Tyner AL, et al. Oncogene 2004;23(23):4173–6.
[65] Conzen SD, Gottlob K, Kandel ES, Khanduri P, Wagner AJ, O'Leary M, et al. Mol Cell Biol 2000;20(16):6008–18.
[66] Hemann MT, Bric A, Teruya-Feldstein J, Herbst A, Nilsson JA, Cordon-Cardo C, et al. Nature 2005;436(7052):807–11.
[67] Thomson JM, Newman M, Parker JS, Morin-Kensicki EM, Wright T, Hammond SM. Genes Dev 2006;20(16):2202–7.
[68] Zhang B, Pan X, Wang Q, Cobb GP, Anderson TA. Comput Biol Chem 2006;30(6):395–407.
[69] Megraw M, Sethupathy P, Corda B, Hatzigeorgiou AG. Nucleic Acids Res 2007;35(database issue):D149–55.
[70] Stark A, Brennecke J, Russell RB, Cohen SM. PLoS Biol 2003;1(3):E60.
[71] Sood P, Krek A, Zavolan M, Macino G, Rajewsky N. Proc Natl Acad Sci U S A 2006;103(8):2746–51.
[72] Chen PY, Manninga H, Slanchev K, Chien M, Russo JJ, Ju J, et al. Genes Dev 2005;19(11):1288–93.
[73] Shamovsky I, Ivannikov M, Kandel ES, Gershon D, Nudler E. Nature 2006;440(7083):556–60.
[74] Kandel ES, Stark GR. In: Sehgal PB, Levy DE, Hirano T, editors. Signal Transducers and Activators of Transcription (STATs): Activation and Biology. The Netherlands: Kluwer Academic Publishers; 2003.
[75] Tam W, Ben Yehuda D, Hayward WS. Mol Cell Biol 1997;17(3):1490–502.
[76] Martin MM, Lee EJ, Buckenberger JA, Schmittgen TD, Elton TS. J Biol Chem 2006;281(27):18277–84.