MicroRNAs in biological processes and carcinogenesis

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MicroRNAs (miRNAs) encoding small non-coding RNAs have been recognized as a very large gene family present in most organisms. The precise biological effects of miRNAs are yet to be elucidated in detail, partly because each miRNA is believed to negatively regulate the expression of hundreds of target genes. Nevertheless, recent findings indicate that carcinogenic processes are associated with alterations in the expression of several miRNAs, suggesting that some function as oncogenes or tumor suppressor genes. The present review focuses on recent findings in this exciting new area of research, with special emphasis on the involvement of miRNAs in cancer development and progression. Further studies are clearly warranted to elucidate the molecular and biological roles of miRNAs, which may ultimately provide both a better understanding of disease development, as well as a foundation for novel strategies for cancer diagnosis and therapy.

Introduction

Small regulatory RNAs, which are increasingly attracting scientific attention, can be divided into two major classes, microRNAs (miRNAs) and small interfering RNAs (siRNAs) (1–4). These two types differ in the nature of their precursor forms, as miRNAs are evolutionally conserved short non-coding RNA molecules generated from precursor transcripts with an imperfectly matched short-hairpin intramolecular structure, whereas siRNAs are synthesized from long perfectly base-paired double-strand RNAs, with RNA-dependent RNA polymerase involvement (Figure 1). Both miRNAs and siRNAs generally interact with miRNAs, and inhibit their expression. Among endogenous siRNAs, repeat-associated siRNAs are derived from repetitive DNA elements, such as transposons and centromeres, and thought to function to promote heterochromatin formation (3). In viral infections, siRNAs derived from virus transcripts exert inhibitory effects on viral infectious processes and virus-derived miRNAs regulate viral infections (5,6). miRNAs have been discovered in plants, animals and associated viruses, whereas the yeast genome is known to lack miRNA genes, suggesting involvement of miRNAs in relatively complex biological processes (1,2,4). Computer-based predictions of the existence of miRNAs initially relied on the presence of sequence conservation. However, recent reports indicate that a large number of non-conserved miRNAs are specific to the human genome, a substantial fraction of which comprises clusters that are frequently adjacent to conserved miRNAs (7,8). The number of miRNAs in the human genome is likely at least 400, and possibly as high as around 1000 (7–12). The large estimated number of miRNAs present in higher mammals may suggest their potential roles in more complex fine-tuning of gene expression in relation to the evolution of complexity (7).

In addition, a new class of mammalian small RNAs, termed Piwi-interacting RNAs (piRNAs), which are preferentially expressed in the testes, have recently been identified, though their biogenesis as well as physiological and pathological functions remain to be determined (13).

The first recognized miRNAs are lin-4 and let-7, which were genetically identified as heterochronic genes controlling the developmental timing of cell fate at larval stages in Caenorhabditis elegans (14–16). It has been shown that lin-4 is upregulated in the first larval (L1) stage, and suppresses expression of the lin-14 and lin-28 gene products by binding to 3′-UTR regions, whereas let-7 expression is induced at the L3/L4 transition in association with suppression of its target, lin-41. lin-4 and let-7 mutants reiterate L1 and L4 stages, respectively. Subsequently, additional biological properties were found to be regulated by other miRNAs. For example, the miRNAs lsy (lateral symmetry defective mutant)-6 and miR-273 control neuronal left/right asymmetry of gene expression in bilateral taste receptor neurons in C.elegans by repressing a homeobox gene, cog-1, and a lsy-6-activating transcription factor, die-1, respectively (17). In addition, some Drosophila miRNAs control neuronal left/right asymmetry of gene expression by inhibiting expression of the apoptotic effector caspase Drice, while another miRNA, bantam, stimulates cell proliferation and prevents apoptosis through inhibition of the pro-apoptotic gene Hid (18). It should be noted that it is conceivable that miRNAs negatively regulate thousands of target miRNAs based on mismatch-permissive recognition of short stretches in 3′-UTR regions, with each target miRNA appearing to be controlled by multiple miRNAs. The expected outcome would therefore be very broad and complex, making it possible to fine-tune gene expression profiles.

Biogenesis of miRNAs

miRNAs function as ~22 nt small RNAs (mature forms) generated by two cleavage steps accomplished by two distinct complexes between an RNase III enzyme and double-strand RNA-binding domain proteins, i.e. Drosha-DGCR8 (DiGeorge syndrome critical region gene 8, a human...
Fig. 1. miRNA biogenesis and silencing mechanisms. miRNAs are initially transcribed as long primary miRNAs (pri-miRNAs) and cleaved at hairpin-stems by the Drosha/DGCR8 microprocessor complex, resulting in pre-miRNAs in the nucleus. After nuclear export, pre-miRNAs are cleaved into miRNA duplexes by Dicer/TRBP, and the guide strand of the duplexes is incorporated into RISC. These two steps may be coupled, because Dicer/TRBP is associated with Ago2, a key component of RISC. miRNAs are thought to regulate about 30% of the genes in the human genome by multiple mechanisms, and their targets include both oncogenes and tumor suppressor genes. miRNAs suppress translation at the initiation and elongation steps, and may also induce deadenylation, decapping and degradation of target mRNAs within P-bodies (GW-bodies).
homolog of Pasha) and Dicer-TRBP [Tar (HIV-1) RNA-binding protein] (Figure 1). miRNAs, which are often present within the introns of usual protein-encoding genes, are initially transcribed as long primary miRNAs (pri-miRNA). All pri-miRNAs analyzed thus far contain a 7-methyl guanosine cap and a poly(A) tail, as with protein-encoding genes, suggesting transcription by RNA polymerase II (19,20). The mature miRNA sequence within a stem region of a short stem–loop structure in a pri-miRNA is thought to be recognized and cleaved at a hairpin-stem by the Drosha-DGCR8 microprocessor complex (21–24), resulting in generation of a ~70 nt-long hairpin-shaped precursor (pre-miRNA) in the nucleus. After subsequent nuclear export by the RanGTP-dependent dsRNA-binding protein Exportin 5 (25–27), Dicer then cleaves pre-miRNA at a position 22 nt from one end, resulting in a ~22 nt double-stranded miRNA duplex (28,29). As is the case with siRNA, this final cleavage process by Dicer may be coupled with specific incorporation of the guide strand of the miRNA duplex into RNA-induced silencing complex (RISC). Dicer interacts with a dsRNA-binding domain protein TRBP, which interacts with a key component of RISC, Argonaute 2 (Ago2; also called translation initiation factor 2C2), and forms a ternary complex of Dicer/TRBP/Ago2. It has been shown that TRBP is required for the recruitment of Ago2 to miRNA/ siRNA bound by Dicer (30,31) and that the PAZ domain of Ago2 may be involved in binding to miRNA, whereas another conserved domain of Ago2, the PIWI domain, acts as a RNAse H-like catalytic center of RISC for siRNA-mediated mRNA cleavage. Dicer has been implicated in heterochromatin maintenance and centromeric silencing (32–34). A cluster of three argonaute genes, Ago1, Ago3 and Ago4, resides within a genomic region frequently affected in Wilms tumors (35), and aberrant expressions of Ago1 and PIW1L1 (human homolog of Drosophila piwi) have also been reported in some tumors (35,36).

Multiple mechanisms involved in miRNA-mediated silencing of gene expression

The initial study demonstrated that lin-4 reduced the level of lin-14 protein without reducing the expression level of lin-14 mRNA, suggesting that miRNAs exert gene silencing by inhibiting translation of target genes (15,37). Consistent with this notion, Ago2-null cells show intact translational repression by mismatched siRNA, however, among human and mouse Argonaute families, only Ago2 possesses mRNA catalytic activity and participates in cleavage-dependent RNA degradation (38). It has been suggested that full translational repression by miRNA may be dependent on the presence of a proper cap structure and poly(A) tail (39,40). The cap binding protein EIF4E and the poly(A) tail binding protein associate with each other through the EIF4G protein complex, which recruits ribosomes. miRNA may disrupt the closed structure of target mRNA, possibly by inhibiting EIF4E function (40). However, a contradictory report has indicated that some cap-independent translation is initiated from an internal ribosomal entry site, which initiates translation independent of initiation factors that can be repressed by miRNA (41). This finding suggests that miRNAs may also repress translation after translation initiation, possibly through induction of premature ribosome drop-off, resulting in the inhibition of translation elongation (41). Therefore, though it has become clear that translational repression is a major silencing mechanism of miRNA, the precise processes involved require further elucidation.

It is noteworthy that miRNAs now appear to affect gene silencing through other mechanisms such as endonucleolytic cleavage and cleavage-independent degradation of the targeted mRNA. A recent study of C. elegans indicated that target mRNA levels are reduced even by the archetypal miRNAs, lin-4 and let-7 (42). Recent analysis also revealed that RISC components, AGO1/2, miRNAs and targeted mRNAs, are localized within cytoplasmic processing bodies (P-bodies), also referred to as GW-bodies derived from GW182, a key subunit of these structures (Figure 1) (39,43,44). P-bodies concentrate the decapping complex DCP1/DCP2 and the 5′–3′ exonuclease XRN1, which are involved in mRNA turnover. The RNA-binding proteins GW182 and DCP1/DCP2 were shown to be required for miRNA-mediated gene silencing (45), suggesting a crucial role for P-body components in the miRNA pathway, and miRNA-mediated induction of decapping and degradation of targeted mRNAs (Figure 1). The Argonaute proteins physically interact with GW182, whose silencing relocates resident P-body proteins and impairs silencing by miRNA (46). Jing et al. (47) recently suggested that the miRNA machinery components Dicer and Ago are indispensable in mRNA decay directed by an AU-rich element in 3′-UTR (ARE), and that human miR-16, which is complementary to ARE, is also required for such ARE-RNA decay, suggesting functional linkage between mRNA decay and miRNA machinery. Therefore, the accumulation of miRNA and target mRNA complexes into P-bodies suggests that sequestration, decapping and degradation of target mRNA may be involved in miRNA-mediated gene silencing. In addition, miR-125b and let-7 have been shown to expedite poly(A) tail removal as an initial step in the accelerated degradation of mRNAs containing elements with which they are imperfectly complementary (48). One possible scenario of miRNA-mediated mRNA accumulation in P-bodies involves deadenylation of target miRNAs, resulting in the disruption of the closed mRNA structure and exposure of the cap-EIF4E complex. This then causes interaction with a P-body component, EIF4E-transporter (EIF4E-T), resulting in sequestration of the target mRNAs (49).

In plants, miRNAs induce cleavage and subsequent destruction of target mRNAs because of nearly complete base pairing (50). In addition, mouse HOXB8 mRNA has been shown to undergo miR-196-directed cleavage according to conserved nearly perfect base pairing (51). Thus, multiple mechanisms appear to be involved in miRNA-directed gene silencing.

Targets of miRNA and evolution of 3′-UTR

The human genome encodes hundreds of miRNA genes, which negatively regulate the expression of protein-coding genes. However, to date, only a small number of target genes have been identified and experimentally verified. The prediction and validation of miRNA target genes are prerequisites for better understanding of all of the inhibitory networks and biological functions of miRNAs. Target gene prediction programs have been developed based on scoring sequence-complementarity between mature miRNA and target sites in the database of 3′-UTRs, as well as the free-energies of the
miRNA–mRNA duplex and conservation of target site sequences among related genomes (52–55). The functional importance of 5’ ‘seed’ sequences have been indicated by prediction algorithms based on the sequence conservation among species (52,53), as well as by systematic mutation experiments (56–58). Most target sites appear to be recognized by perfect complementation with the 6–8 nt-long ‘seed’ sequence at the 5’ portion of a mature miRNA, and additional base pairing at the 3’ portion is not necessarily required for efficient repression. In contrast, some target sites [e.g. let-7 site in lin-41 (59)] are recognized by imperfect 5’ matches in combination with compensatory additional base pairing at the 3’ end (58). A 3’-UTR analysis in a comparative genomics study searching for functional elements has indicated that previous estimates of the number of human miRNA genes are low and that miRNAs regulate at least 20% of human genes. (10).

Along with those analyses, several programs to predict miRNA target genes in the human genome have been developed, including DIANA-microT (57), miRanda (60), TargetScanS (61) [improved version of TargetScan (53)] and PicTar (62). The TargetScanS program is mainly based on shorter 6mer seed sequences without free-energy calculation, while miRanda and PicTar score sequence-complementarity for both the 5’ and 3’ portions of mature miRNA, free-energies of the miRNA–mRNA duplex, and evolutionary conservation of the target site. Further, PicTar can predict target sites co-regulated by several miRNAs in a coordinated manner.

Short seed sequences have been conserved in families of miRNAs and are present in a number of miRNAs. It has been predicted that each miRNA controls the expression of hundreds of target mRNAs and that each mRNA is targeted by multiple miRNAs. As a result, it has been suggested that up to 30% of genes may be regulated by miRNAs in mammalian cells (61,62). Multiple miRNA-binding sites are required for efficient repression of target mRNA, whereas a single perfectly identical site may be sufficient for RNAi processes. In addition, target genes with different numbers of target sites at 3’-UTR may be regulated to a different extent by the same miRNAs (63). Recent findings indicate that widespread inhibition of target genes occurs in tissue-specific and developmental stage-specific manners (64). It is interesting to note that analysis of miRNA target site predictions indicates that genes with more miRNA sites contain a longer 3’-UTR with a higher density of miRNA sites and that these genes are mainly involved in developmental processes. In contrast, genes with few sites often have a shorter 3’-UTR with lower density and tend to be involved in basic cellular processes (65). These findings suggest a widespread contribution of miRNAs to developmental stage-specific or tissue-specific mRNA expression in relation to the evolution of 3’-UTR (64–66).

The ultimate effects of inhibitory regulatory networks involving miRNAs are expected to be very broad and complex, and show a tissue/cell-specific lineage. In addition to bioinformatic analyses, genome-wide searches for miRNA targeted genes have been conducted using microarray-based expression profiling analysis of cells transfected with mature miRNAs, with the results indicating that each miRNA regulates steady-state levels of hundreds of mRNAs (67). Proteomic comparison between the wild-type and Dicer-1-mutant also suggested the presence of a miRNA-mediated tight control of genes related to protein biogenesis and turnover during Drosophila oocyte maturation (68). Additional studies using overexpression or silencing of individual miRNAs combined with systematic analysis of transcriptome and/or proteome should provide further insight into the regulatory networks of miRNAs, as well as their precise roles in biological and pathological processes.

Biological roles of miRNAs in mammalian cells

The biological roles of miRNAs in mammalian cells are not well understood at the present, though there are several examples for which functions have been elucidated to a considerable extent. miR-1 and miR-133a constitute two clusters (miR-1-2/miR-133a-1 and miR-1-1/miR-133a-2), which are transcriptionally induced by serum response factor (SRF) and MyoD. Interestingly, these miRNAs are transcribed together, yet carry out distinct and conflicting biological functions during skeletal muscle development. miR-1 targets HDAC4, an inhibitor of MyoD/MEF2 complex formation, resulting in the promotion of myogenesis differentiation, whereas miR-133a represses SRF, consequently abrogating its inhibition of myoblast proliferation. Interestingly, HDAC4 and SRF in turn regulate transcription of the miR-1-3-133a cluster, though in different manners. Therefore, these muscle-specific miRNAs participate in complex transcriptional circuits that conceivably fine-tune skeletal muscle proliferation and differentiation (69). miR-1 also targets the transcription factor Hand2, which may help maintain an adequate pool of undifferentiated myocyte precursors during cardiogenesis (70).

Chen et al. (71) previously showed that ectopic expression of miR-181 in hematopoietic stem/progenitor cells led to an increased fraction of B-lineage cells. Fazi et al. (72) also recently noted that miR-223 may govern granulocytic differentiation by targeting the NFI-A transcription factor, which in turn negatively regulates C/EBPβ-mediated miR-223 expression through its competitive binding to the promoter region of miR-223, indicating a crucial role of C/EBPβ-miR-223-NFI-A signaling circuitry in granulopoiesis. Considerable evidence has also accumulated regarding the roles of miRNAs in neuronal and neural cell differentiation. For example, induction of neuronal differentiation upregulated miR-125b (a mammalian lin-4 homolog) and downregulated lin-28 mRNA in vitro (73), while the brain-specific miRNAs miR-124a and miR-9 have been shown to affect neural lineage differentiation in ES cell derived cultures (74). In addition, the zinc-finger transcriptional repressor RE1 silencing transcription factor (REST), which inhibits the expression of neuronal genes in non-neuronal cells, directly represses brain-specific miRNAs, including miR-124a and miR-9. It has also been shown that during in vitro and in vivo neuronal differentiation, REST is downregulated, resulting in the induction of neuronal genes and brain-specific miRNAs (75).

miRNAs in carcinogenesis

Recent findings indicate that alterations in the expression of several miRNAs are often present in human cancers, suggesting potential roles of miRNAs in carcinogenic processes. Expressions of some miRNAs, such as let-7 (76), the miR-15a/miR-16-1 cluster (77,78) and neighboring
miR-143/miR-145 (79), have been reported to be reduced in some malignancies, suggesting their potential tumor suppressor activities. In contrast, some other miRNAs, such as the miR-17-92 cluster (80–82) and miR-155/BIC (83–86), are known to be overexpressed, suggesting their oncogenic potentials. In addition, some miRNAs with altered expression levels appear to be associated with certain genetic alterations, such as deletion, amplification and mutation. In the following sections, alterations of miRNAs in human cancers thus far reported are summarized in detail.

Involvement of archetypical miRNAs, let-7 and lin-4, in human cancers

Emerging evidence suggests that an archetypical miRNA, let-7, may be involved in human carcinogenesis. We have reported the frequent occurrence of significantly reduced expression of family members of the let-7 miRNA genes in lung cancers in association with shortened postoperative survival, as well as a modest reduction of cell growth by forced expression of let-7 in a lung cancer cell line expressing let-7 at a low level (76). The prognostic significance of a reduced expression of let-7 in lung cancer was also confirmed in a recent expression profiling analysis of miRNAs (87). These notions of the potential biological and clinical roles of altered miRNA expression in human cancers are further supported by the identification of RAS as a target gene for let-7. In C. elegans, the let-7 family negatively regulates let-60 gene encoding of small GTPases (homologs of RAS oncogenes), while loss of let-60/RAS suppresses the let-7 mutant phenotype. Further, the 3′-UTR of let-60/RAS contains multiple let-7 complementary sites. Johnson et al. (88) found that the human RAS gene also contains multiple let-7 complementary sites and is indeed regulated by let-7, providing clues to a mechanistic explanation for let-7 alterations in human lung cancer. Characterization of a vertebrate homolog of lin-41, a target gene of let-7, has shown that it contains multiple let-7 target sites, suggesting a role in embryogenesis, especially in limb development (89,90). Virtual extinction of lin-41 expression occurs at later developmental stages (90). Involvement of the LIN41 gene in tumorigenesis has yet to be clearly demonstrated, however, the human LIN41 gene is present in the 3p23 region, which is frequently deleted in lung cancers.

Another archetypical miRNA, lin-4, may contribute to human carcinogenesis. In C. elegans, lin-14, a target of lin-4, is a transcription factor that regulates several downstream targets including the insulin/insulin-like growth factor family gene ins-33 (91). Interestingly, it has been shown that reduction of the activity of lin-4 shortens life span and accelerates tissue aging, whereas overexpression of lin-4 or reduction of lin-14 activity extends life span through regulation of the insulin-like growth factor-1 receptor pathway (92). Although a vertebrate homolog of lin-14 has yet to be identified, another target of lin-4, lin-28, does exist in mammals including humans, and encodes RNA-binding Zinc finger proteins (93). miR-125b-mediated downregulation of lin-28 has been suggested to contribute to neuronal differentiation in mouse P19 embryonal carcinoma cells (73), while depletion of miR-125b was found to have profound inhibitory effects on the proliferation of adult differentiated cancer cells, which was rescued by co-transfected mature miR-125b (94). It is of note that insertion of miR-125b-1 into a rearranged immunoglobulin heavy chain gene locus was recently reported in a patient with precursor B-cell acute lymphoblastic leukemia, though the expression miR-125b-1 was not investigated (95). Thus, available findings suggest potential roles of miR-125b-1 in carcinogenic processes, though their significance must be confirmed in further studies.

miRNAs in cancer-associated genomic regions

Calin et al. (96) noted that miRNAs are frequently located in fragile sites as well as in cancer-associated genomic regions, such as minimal regions with a loss of heterozygosity or amplification, or at common breakpoints. In fact, a miRNA cluster comprising miR-15a and miR-16-1 at chromosome 13q14 was the first such example reported to have altered expression in cancer cells. Detailed analyses of deletion and expression status revealed these two miRNAs to be located within a 30 kb minimally deleted region in chronic lymphocytic leukemia (CLL) patients, both of which were deleted or downregulated in most CLL cases, suggesting that miR-15a and miR-16-1 may function as tumor suppressor genes (77). It was later shown that these miRNAs share target genes, together with miR-195, while among the predicted targets, BCL-2 mRNA is a possible crucial target with regard to leukemogenesis (97). Therefore, it is possible that upregulation of BCL2 by reduced expression of these miRNAs contributes to the pathogenic state of CLL, because BCL2 functions as an apoptotic suppressor and promotes lymphomagenesis. Downregulation of miR-15 and miR-16 miRNAs also appears to be a feature of pituitary adenomas (78). Further, it is noteworthy that a germ-line mutation of miR-16-1 was found in two CLL patients, one of whom was member of a family that fulfilled the minimal criteria for familial CLL, suggesting that germ-line/somatic mutations in miRNA genes play a role in cancer development (98). However, the roles of alterations of the miR-15a/miR-16-1 cluster in the development and progression of leukemia might be more complicated than initially thought, and require elucidation through further experimentation. For example, CLL patients with deletions of 13q14, where miR-15a and miR-16-1 reside, have been shown to exhibit a more favorable prognosis (99), and Calin et al. (98) reported that high expression levels of miR-16-1/miR-16-2 were observed in CLL patients with a poor prognosis. A paralogous cluster comprising miR-15b and miR-16-2 is localized at 3q25, a chromosomal region known to be amplified in several types of cancers (100). This contrasts with the report of homozygous deletions in lung cancers (101).

The human genome has three paralogous polycistronic miRNA clusters (miR-17-92 at 13q31.3, miR-106a-92 at Xq26.2 and miR-106b-25 at 7q22) that carry similar constituents. Suggestive evidence for the involvement of the miR-17-92 cluster at 13q31.3 in B-cell lymphoma was first obtained through a detailed array CGH analysis by Ota et al. (102) who suggested that the C13orf25 gene, which encodes a short ORF and includes the miR-17-92 cluster, may be the target for this amplification. This finding prompted He et al. (80) to study this genomic region from a miRNA point of view, which resulted in identification of overexpression of mature miRNAs derived from the miR-17-92 cluster and an association with gene amplification in B-cell lymphomas (81). Importantly, enforced expression of this polycistronic cluster may accelerate B-cell lymphoma development in
miRNA cluster also appears to be a direct target for translational inhibition by miR-17-5p and miR-20 residing in this cluster (103), and E2F1 displays the properties of both an oncogene and tumor suppressor. E2F1 is also able to advance quiescent cells into the S phase, and induce apoptosis in p53-dependent and p53-independent pathways (104). Therefore, the repression of E2F1 by miR-17-92 might contribute to cancer development. E2F1 has been shown to be a direct target for translational inhibition by miR-17-5p and miR-20 in in silico predictions (60,84), suggesting that the signaling cascade from transcriptional factor-type oncogenes to cancer-associated miRNAs may participate in cancer developments. E2F1 has been shown to be involved in adipocyte differentiation (107), possibly through regulation of a predicted target gene ERK5 (MAPK7), which is known to promote cell growth and proliferation in response to tyrosine kinase signaling. The chicken non-coding RNA gene Bic was originally identified as a proviral insertion site in avian lymphomas and chicken oncogenicity assays have indicated its cooperation with Myc in lymphomagenesis (108). However, Bic lacks a conserved ORF, in contrast to evolutionary conservation of the RNA secondary structure (109), while the conserved region at the C-terminal exon encodes the miR-153 gene. miR-153/BIC accumulates during activation of B- and T-cells as well as in Hodgkin lymphomas (83), B-cell lymphomas (84) and subsets of Burkitt lymphoma (85,86). According to in silico predictions (60,84), miR-153/BIC may target the transcriptional factors PU.1 and C/EBPβ, both of which control differentiation of B-cells (110). Interestingly, Eμ-murine-miR-115 transgenic mice initially exhibit preleukemic pre-B-cell proliferation, which is subsequently followed by frank B-cell malignancy probably through secondary genetic alterations, suggesting the ability of miR-153 to induce polyclonal expansion of B-cell lineage cells (111). In human primary lung fibroblasts, miR-155 binds to the 3′-UTR of the c-Myc transgenic mice by reducing apoptosis, further strengthening the notion of miR-17-92 involvement. This miRNA cluster also appears to be a frequent target for genetic abnormalities in common cancers in adults. In a search for miRNAs with altered expression in lung cancers, we independently identified miRNAs at the miR-17-92 cluster that were markedly overexpressed, sometimes with increased numbers of gene copies (82). miR-17-92 miRNAs are preferentially overexpressed in small cell lung cancers characterized by neuroendocrine differentiation, suggesting that histologic type-related transcription factors might be involved. Further, the miR-17-92 cluster has been suggested to be important for embryogenesis (64). Our finding that introduction of the miR-17-92 cluster, but not the putative ORF of the C13orf25 gene, enhanced lung cancer cell growth (82), lends further credence to a contribution of highly conserved miRNAs rather than a short non-conserved ORF.

Involvement of other miRNAs in carcinogenesis

A growing number of miRNAs have been suggested to play roles in human carcinogenesis with different levels of certainty. Reduced steady-state levels of two neighboring miRNAs, miR-143 and miR-145, may be a feature of adenomatous polyps and cancers of colorectal epithelium (79), while miR-143 has been shown to be involved in adipocyte differentiation (107), possibly through regulation of a predicted target gene ERK5 (MAPK7), which is known to promote cell growth and proliferation in response to tyrosine kinase signaling.

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### Table I. Alteration of miRNA expression in human malignancies

<table>
<thead>
<tr>
<th>Altered miRNA</th>
<th>Locus</th>
<th>Cancer typea</th>
<th>Directly regulated targets</th>
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<tbody>
<tr>
<td>Downregulated (candidate tumor suppressor genes)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>let-7 family</td>
<td>Multiple</td>
<td>Lung (76,87) associated with poor survival</td>
<td>Ras family (88)</td>
</tr>
<tr>
<td>miR-15a/16 (in the intron of DLEU2 gene)</td>
<td>13q14.2</td>
<td>B-CLL (77), germ-line mutations in B-CLL (98); pituitary (78)</td>
<td>BCL2 (97)</td>
</tr>
<tr>
<td>miR-143/145</td>
<td>5q32</td>
<td>Colorectal (79); (miR-145) breast (122)</td>
<td>(miR-143) ERK5 (MAPK7) (107)</td>
</tr>
<tr>
<td>miR-125b (lin-4)</td>
<td>11q24.1 (125b-1)</td>
<td>Breast (122)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>21q21.2 (125b-2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upregulated (candidate oncogenes)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-17-92 (in C13orf25 gene)</td>
<td>13q31.3</td>
<td>BCL with gene amplification (80,81,102) lung (82); (miR-17-5p) breast, colon, lung, pancreas, prostate (106); (miR-18) HCC (123)</td>
<td>E2F1 (miR-17-5p, miR-20) (103), TGFβRII (miR-20) (106)</td>
</tr>
<tr>
<td>miR-106a</td>
<td>Xq26.2</td>
<td>Colon, pancreas, prostate (106)</td>
<td>RB-1 (106)</td>
</tr>
<tr>
<td>miR-155 (in non-coding BIC gene)</td>
<td>21q21.3</td>
<td>HL (83); BCL (83,84); pediatric BL (85,86); breast (122); lung, associated with poor survival (87)</td>
<td>ND</td>
</tr>
<tr>
<td>miR-221/222</td>
<td>Xp11.3</td>
<td>Papillary thyroid carcinoma (114); glioblastoma (128)</td>
<td>KIT (113)</td>
</tr>
<tr>
<td>miR-21</td>
<td>17q23.2</td>
<td>Glioblastoma (115,128); breast (122); breast, colon, lung, pancreas, prostate, stomach (106)</td>
<td>ND</td>
</tr>
<tr>
<td>miR-372/373</td>
<td>19q13.42</td>
<td>Testicular germ cell tumor (116)</td>
<td>LAT52 (116)</td>
</tr>
<tr>
<td>miR-191 (in the intron of overlapping DALRD3 and C3orf60 genes)</td>
<td>3p21.31</td>
<td>Colon, lung, pancreas, prostate, stomach (106)</td>
<td></td>
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</tbody>
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*aCLL, chronic lymphocytic leukemia; BCL, B-cell lymphoma; HL, Hodgkin’s lymphoma; BL, Burkitt lymphoma; DLEU2, deleted in lymphocytic leukemia 2.

bND, not determined.
of angiotensin II type 1 receptor (AGTR1) mRNAs and represses its expression, while TGF-β1 treatment results in a decreased expression of miR-155 and increased expression of AGTR1, suggesting that these changes in expression might be related to the growth-inhibitory effects of TGF-β1 (112).

During erythropoietic differentiation of CD34+ hematopoietic progenitor cells (HPCs), miR-221 and miR-222 have been shown to be gradually but markedly downregulated, and that downmodulation was inversely related to an increased expression of kit protein, which is a key molecule to regulate HPC proliferation (113). Both miRNAs interact with the 3′-UTR of Kit mRNA and repress its expression. In fact, introduction of miR-221 and miR-222 inhibited the engrafment capacity and stem cell activity of CD34+ HPCs, and also inhibited proliferation of an erythroleukemia cell line with Kit expression (113). Another report that used miRNA microarray analysis of papillary thyroid carcinomas (PTC) also demonstrated upregulation of miR-221 and miR-222, as well as miR-146 (114). While miR-146 is predicted to interact with KIT mRNA 3′-UTR, upregulation of these three miRNAs appears to be associated with reduced KIT expression in PTC (114). Interestingly, two SNPs located in miRNA-interacting sites within the KIT mRNA appear to have an influence on the effects of miRNAs (114).

Another example of overexpression of an miRNA is provided by glioblastomas. miR-21 is strongly overexpressed in this highly malignant brain tumor type, while knockdown of miR-21 in glioblastoma cells by an antisense-oligonucleotide triggered activation of caspases and led to increased apoptotic cell death, suggesting that miR-21 overexpression may contribute to the malignant phenotype by suppressing critical apoptosis-related genes (115).

Voorhoeve et al. (116) employed a novel strategy by combining an miRNA vector library and corresponding bar code array in order to identify miRNAs functionally associated with carcinogenesis. They introduced the miRNA library into primary cells expressing RASV12, which normally fall into oncogene-induced premature senescence in a p53 dependent manner, and searched for miRNAs that could overcome the senescence barrier. miR-372 and miR-373 were consequently found to permit proliferation and tumorigenesis of these primary cells carrying both oncogenic RAS and wild-type p53, probably through direct inhibition of the expression of the tumor-suppressor LATS2 and subsequent neutralization of the p53 pathway. Interestingly, these miRNAs were found to be abundantly expressed in testicular germ cell tumors retaining wild-type p53.

In addition to alternations in miRNAs themselves, changes in miRNA-processing mechanisms may play a role in the development of cancers. In this regard, it is interesting to note that Harris et al. (117) recently reported that Dicer may be essential for lung epithelium morphogenesis, probably by imposing proper regulation of FGF10 expression. We also observed that a reduced expression of Dicer was correlated with shorter survival of lung cancer patients, suggesting a possible link with lung cancer progression (118).

**Diagnosis and prognostic prediction using miRNA expression signature**

Global expression profiling analysis of protein-coding genes has provided deep insight into cancer biology and has been shown to be novel means for establishing cancer diagnoses. In initial studies, quantification of mature miRNAs was conducted by northern blot analysis, though that is obviously not an ideal method to comprehensively analyze expression profiles, because of the low-throughput and specificity in terms of discrimination of various isoforms. Accordingly, microarray or bead-based techniques are increasingly being applied for genome-wide assessment of expression profiles of mature miRNA forms. It is now clear that there are tissue-specific miRNA expression signatures, suggesting a relevance with tissue differentiation (119). Expression profiling analysis of various cancers has also revealed alterations in miRNA levels and, interestingly, those aberrations more frequently feature downregulation than upregulation, possibly reflecting differentiation defects in cancer cells. It has also been shown that specific signatures may exist for each cancer type, suggesting their utility for cancer classification and even prognosis prediction. Lu et al. (120) compared global expression profiles between miRNAs and mRNAs, and reported the former to be superior for tumor-type classification with poorly differentiated cancers. It is possible that this superiority of miRNA profiling may reflect the inherent close relationship with developmental lineage and differentiation state.

Another recent microarray-based large-scale profiling analysis of 540 normal and tumor samples from six types of tissues (lung, breast, stomach, prostate, colon and pancreas) demonstrated a solid cancer miRNA signature (106). In contrast to the report by Lu et al. (120), most of the miRNAs picked up in that study showed upregulation in cancers. It is not clear at the present time whether this apparent discrepancy may have resulted from use of different technical platforms, or differences in the sample numbers and tissues analyzed. The fact that some of the miRNA levels were high in all or most of these tumors may imply their general role in oncogenesis. The list of differentially expressed miRNAs contains well-characterized cancer-associated miRNAs, including miR-17-5p, miR-20a, miR-21, miR-92, miR-106a and miR-155. Strikingly, miR-21 was significantly overexpressed in tumors from all six tissue types, while miR-17-5p and miR-191 were overexpressed in tumors from five (excluding those of the stomach and breast, respectively), suggesting the general roles of these three miRNAs in carcinogenesis. The predicted targets for the differentially expressed miRNAs are significantly rich in genes encoding tumor suppressors and oncoproteins. miR-106 and miR-20a inhibit the retinoblastoma (RB1) and TGFβRII genes, respectively, and miR-26a-1 targets the PLAG1 putative oncogene (106). Therefore, it is conceivable that alterations of the expression of these miRNAs may play roles in carcinogenesis. miRNA expression profiling analysis of lung cancers indicates the presence of miRNAs differentially expressed between lung cancers and corresponding non-cancerous tissues, or between adenocarcinomas and squamous cell carcinomas (87). miRNA expression was also shown to correlate with the prognosis of adenocarcinoma patients, especially in the case of three miRNAs (miR-155, miR-17-3p and miR-20). In addition, a high expression of miR-155 has been suggested to be significantly associated with unfavorable prognosis in lung adenocarcinoma patients (87).

The same research group also reported that a unique expression signature composed of 13 miRNA genes could distinguish CLL cases with a poor prognosis and the high-ZAP-70/unmutated IgVH phenotype from those with a favorable prognosis and low-ZAP-70/mutated IgVH.
Cancer therapy by manipulation of miRNAs

Rapidly accumulating evidence from recent studies suggests that miRNAs might be viable therapeutic targets for a wide range of diseases, including cancer. For example, antisense 2'-O-methyl oligoribonucleotides may specifically inactivate corresponding miRNAs in human cells (124,125). The induction or direct introduction of miR-221 and miR-222 may inhibit proliferation of erythroleukemia cells expressing KIT protein (113), while efficient and specific silencing of endogenous miRNAs such as miR-21, which is achievable with the aid of an antisense oligonucleotide specific to a loop sequence of pre-miRNA, might be useful in the treatment of glioblastomas (115). We have noted that inhibition of miR-17-5p and miR-20a with antisense oligonucleotides can induce apoptosis selectively in lung cancer cells overexpressing the miR-17–92 cluster, suggesting the possibility of ‘OncomiR addiction’ in a subset of lung cancers (Matsubara H., Osada H. et al., manuscript submitted). It is of note that Krtutzfeldt et al. (126) recently reported that intravenous administration of a chemically modified cholesterol-conjugated single strand RNA complementary to a mature miRNA, an ‘antagomir,’ could markedly inhibit miRNA expression in most organs other than the CNS. Administration of an antagonir for miR-122, an abundant liver-specific miRNA, resulted in upregulation of genes with 3' UTR miR-122 recognition motifs, leading to a reduction in plasma cholesterol levels. These findings suggest potential future therapeutic applications of miRNA silencing in several diseases including cancers. Functional screening for miRNAs involved in the regulation of cell growth or death with a library of miRNA inhibitors may also provide a base for establishing novel miRNA-based therapies (127). Also, overexpression of miRNAs with tumor suppressor functions such as the let-7 family might be a useful strategy to control tumor growth (76).

Conclusion

The significance and broad roles of miRNAs in several biological processes, including embryogenesis, differentiation, proliferation and apoptosis, as well as carcinogenesis, have become clear. While evidence for the presence of alterations of miRNAs in human cancers has also been rapidly accumulating, detailed information obtained through further studies is still needed, including genome-wide expression profiling analysis of miRNAs in conjunction with expression profiles of mRNAs of protein-coding genes and gene products, as well as elucidation of the molecular and biological consequences of miRNA alterations in each cancer type. Future reporting of in-depth knowledge in this important and newly expanding area of cancer research may lead to establishment of novel methods for precisely classifying human cancers based on miRNA expression profiles. In addition, future results may ultimately result in the development of novel therapeutic strategies, allowing efficient techniques to comprehensively modulate mRNA expression profiles that would hopefully have profound effects on cancer cell growth and survival. Finally, though it certainly appears that a new frontier of cancer research has been revealed, it seems to extend beyond the area of miRNA research. Further exploration for the abundant information still unknown in regard to the potential involvement of other non-coding RNA species, such as recently found piRNAs, appears to be necessary to fully understand the molecular carcinogenic processes of human cancers. To this end, completion of the human genome project, and recent innovations in experimental and bioinformatics analyses will greatly help to accomplish this exciting and rewarding endeavor.

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References


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