MicroRNAs: Fundamental Facts and Involvement in Human Diseases

Gianpiero Di Leva, George A. Calin, and Carlo M. Croce*

MicroRNAs (miRNAs) are a group of small noncoding RNAs that have been identified in a variety of organisms. These small, 18–22-nucleotide (nt) RNAs are transcribed as parts of longer molecules called pri-miRNAs, which are processed in the nucleus into hairpin RNAs of 70–100 nt, called pre-miRNAs, by the double-stranded RNA (dsRNA)-specific ribonuclease Drosha. The function of most miRNAs is not known, but for a few members the participation in essential biological processes for the eukaryotic cell is proven. In this review, we summarize how miRNAs were discovered, their biological functions, and importance in animal development, highlighting their function in proliferation, apoptosis, and cell differentiation. Furthermore, we discuss the deregulation of miRNAs in human diseases and their involvement in tumorigenesis. Birth Defects Research (Part C) 78:180–189, 2006. © 2006 Wiley-Liss, Inc.

DISCOVERY OF microRNAs

The discovery of microRNAs (miRNAs) began in early 1981 when Martin Chalfie et al. (1981), during a loss-of-function study in C. elegans, revealed that mutations in the lin-4 gene lead to continued synthesis of larval-specific cuticles. At that time, together with lin-14, lin-29, and lin-28, these genes were classified as heterochronic genes, capable to control the timing of specific postembryonic developmental events in C. elegans. Seven years later, Victor Ambros (1989) described, in hypodermal cells, an interaction hierarchy of heterochronic regulatory genes to coordinate the "larva-to-adult switch." These experiments have shown that, in the early stages of development, lin-14 and lin-28 inhibit lin-29, preventing the switch; next, lin-4 inhibits lin-14 and lin-28, triggering the activation of lin-29 and the following switch in the L4 larval stage (Ambros, 1989).

Lin-14 has been shown to encode a nuclear protein that is normally present in most somatic cells of late embryos and L1 larvae, but not in the later larval stages or adults. Gary Ruvkun et al. (1991) found that two lin-14 gain-of-function mutations lead to an abnormal protein accumulation in the later larval stages; these mutations delete the 3′ untranslated region (UTR) of lin-14 mRNA, highlighting a regulatory element in the UTR that controls the temporal gradient of the protein. Since lin-4 was described to downregulate the temporal levels of lin-14 protein, the authors proposed that the lin-4 gene product could be the trans-acting factor, capable of binding the 3′UTR of lin-14 and negatively regulating it. Finally, in 1993, two independent studies, published in the same issue of Cell by Lee et al. (1993) and Wightman et al. (1993), presented the real nature of the lin-4 gene and its ability to regulate heterochronic gene expression. After cloning the lin-4 gene, they demonstrated that the potential open reading frame of lin-4 does not encode for a protein; they identified two small lin-4 transcripts of approximately 22 and 61 nt and found that the 3′UTR of lin-14 mRNA contains sequences complementary to lin-4. These data suggested that the temporal regulation of lin-14 is driven by lin-4 RNA through antisense RNA-RNA interactions, involving the small RNA lin-4 and the 3′UTR of lin-14, whose translation was inhibited. Seven years later, Reinhart et al. (2000) showed that the let-7 gene is another heterochronic switch gene coding for a small 21-nt RNA, with complementary sequence to the 3′UTR of lin-14, lin-28, lin-41, lin-42, and daf-12; they proposed that the sequential stage-specific expression of let-7 and lin-4 RNAs was capable through an RNA-RNA interaction with the 3′UTR of the target genes to trigger the temporal cascade of regulatory heterochronic genes specifying the timing of C. elegans developmental events (Reinhart et al., 2000).

At that time, these discoveries were considered as a new piece in the complicated gene expression regulation puzzle restricted to the small temporal RNA (stRNA) let-7 and lin-4 in worms. This idea was completely changed when independent groups tried to investigate whether RNAs similar to stRNA...
could play a more general role in gene regulation (Lagos-Quintana et al., 2001; Lau et al., 2001; Lee and Ambros, 2001). Cloning the RNA from different organisms and cellular systems, by using the same strategy applied to clone small interfering RNA (siRNA) processed from exogenous double-stranded RNAs (dsRNAs) in an embryo lysate, researchers in three laboratories were able to isolate a new class of RNAs with the same characteristics of lin-4 and let-7, providing evidence for the existence of a large class of small RNAs with potential regulatory roles. Because of their small size, the authors referred to these novel RNAs as microRNAs (miRNAs), a new abundant class of riboregulators that could regulate the gene expression at posttranslational level by base-pairing the 3′UTR of mRNA targets. After this discovery, a new challenge for the researchers was to define the biological function and the potential target genes of these new genes.

miRNAs: GENOMICS, BIOGENESIS, AND MECHANISM OF ACTION

How Many miRNAs Are Enough?

At the beginning, the majority of miRNAs were identified by direct cloning of small RNAs (Bartel, 2004); this approach had constriction because it permitted just the detection of abundantly expressed miRNAs. Three observations suggested that miRNAs identification could be facilitated using computational approaches. First, miRNAs are produced from a precursor transcript of 70–100 nt with a extended stem-loop structure. Second, miRNAs are usually highly conserved between the genomes of related species. Third, miRNAs display a characteristic pattern of evolutionary divergence. In accordance with these criteria, many computational procedures have been developed to predict miRNAs in the genome of different organisms, such as miRNAsScan (http://genes.mit.edu/mirscan) in humans, miRNaseeker (http://www.frutfly.org/seq_tools/miSeeker.html) in Drosophila, or others in C. elegans (for review see Bentwich, 2005; Brown and Sanseau, 2005). The sensitivity of these bioinformatic approaches was demonstrated by the presence of a high percentage of previously experimentally identified miRNAs within the top predicted candidates and by confirmation using Northern blotting analysis and a more sensitive PCR method (Schmidtgen et al., 2004). The estimate of miRNA genes in the metazoan genome represents nearly 1% of the predicted genes, a fraction similar to that seen for other very large gene families with regulatory roles, such as those encoding transcription-factor proteins.

In 2003, the rapid growth of the number of miRNA genes led Sam Griffiths-Jones from the Wellcome Trust’s Sanger Institute to create a comprehensive and searchable database of published miRNA sequences via a web interface: The miRNA Registry (http://microrna.sanger.ac.uk/sequences) (Griffiths-Jones, 2004). The primary aims of this registry are two-fold. The first is to avoid inadvertent overlap by assigning unique names to distinct miRNAs. The miRNAs are annotated with numerical identifiers based on sequence similarity; for example, if a standard name is “miR-15,” the next miRNA without similarity will receive the name “miR-16.” For homolog miRNAs in different organisms, it is usual to assign the same name on the similarity of the 22 nt mature sequence. Identical mature forms are assigned the same name and, if they are produced from different genomic loci, they are differentiated by suffixes such as “miR-16-1” and “miR-16-2.” Differences in one or two bases are identified by suffixes, such as “miR-181a” and “miR-181b.” If an miRNA hairpin precursor gives rise to two mature miRNAs, one from each arm, the rule is to denote miRNAs in the form “miR-142-5p” (5′ arm) and “miR-142-3p” (3′ arm) until the data will confirm which form is predominantly expressed; in such cases, the species less expressed will be identified by using an asterisk (such as “miR-191∗”). The second aim of the miRNA registry is to provide a database for all miRNAs sequences, including the stem-loop structure, with the highlighted miRNA in red, genomic location, homologous sequences, and possible target predictions.

At the beginning of 2005, a phylogenetic shadowing study on miRNAs in primate species revealed a characteristic conservation profile of miRNAs genes that the authors have used to efficiently detect 83% of the known miRNAs and predict an extensive set of novel miRNAs, opening the possibility that as many as 1000 miRNAs may exist in the human genome (Berezikov et al., 2005). In version 8 (February 2006), the miRNA Registry contained 3518 entries from 40 organisms including viruses and mammals; there were 332 human miRNAs and the number is continuously expanding (Griffiths-Jones et al., 2006).

Genomics of miRNAs

Almost 50% of mammalian miRNAs are located in introns of protein encoding genes or long noncoding RNA (ncRNA) transcripts, whereas the remaining part is considered as independent transcription units with specific promoter core elements and polyadenylation signals (for review see Pasquinelli, 2002; Cullen, 2004; Kim and Nam, 2006). Among the intragenic miRNAs, 40% are found in introns of protein encoding genes, whereas ~10% are located in introns of long ncRNA transcripts. The vast majority of miRNA clusters are single transcription units or overlapped in the same host transcripts, within exons or introns, and in some cases depend on alternative splicing of the host gene, implying that they are polycistronic transcripts. Additionally, many miRNAs overlap with two or more transcription units transcribed on opposite DNA strands.

The analysis of the genomic loci of miRNAs demonstrates that host genes encoding proteins are involved in a broad spectrum of biological functions, ranging from embryonic development to the cell
cycle and physiology. When the miRNA host genes are classified for gene ontology (GO) “biological process,” the two most commonly identified biological processes are “metabolism” and “cellular physiological process,” whereas the classification for GO “molecular function” identifies “purine nucleotide binding” and “DNA binding” proteins. Remarkably, several host genes are involved in human disease: the Chloride Channel Protein 5 (CCP5) gene is involved in Dent disease and nephrolithiasis, an X-linked recessive disorder (Frymoyer et al., 1991); this gene hosts miR-188, but a causative role in this disease for miR-188 has not yet been explored.

In addition to the miRNAs located in protein coding genes, a large group of miRNAs resides in transcripts that lack a significant protein-coding potential, classified as long ncRNAs. These types of ncRNA transcripts are sometimes referred to as mRNA-like ncRNAs (mlncRNA) because they are spliced, polyadenylated, and also spatio temporally expressed. Deleted in Leukemia 2 (DLEU2) and BIC are host-genes mlncRNAs, for miR-15a/16-1 cluster and miR-155, respectively (Cai et al., 2002; Eis et al., 2005).

The maturation of miRNAs is a very complex process, and in the following sections we will try to illustrate the machinery that the cell needs to activate the intricate multistep processing from nucleus to cytoplasm, required for the production of miRNAs.

**Transcription and Maturation of miRNAs**

Initially, the researchers believed that miRNAs were transcribed by RNA polymerase III like other small RNAs, and as some transfer RNAs (tRNAs). However, numerous evidence supported the possibility of a transcription mediated by RNA polymerase II. Several polyadenylated transcripts, long kilobases and comprising microRNAs, have been identified in expressed sequence tag (EST) analysis (Tam, 2001; Smalheiser, 2003; Bracht et al., 2004), and the expression profiles of miRNAs and host genes evidenced an elaborate expression control, typical for genes transcribed by RNA polymerase II. In 2004, three direct pieces of evidence were reported to evaluate the strict correlation between miRNAs and polymerase II: 1) the miRNA transcripts are capped and polyadenylated; 2) the transcription of miRNAs is sensitive to a-amanitin at the specific concentration for polymerase II inhibition; and 3) the promoter region, responsible for miRNA transcription, is associated with the polymerase II complex (Kim and Nam, 2006; Lee et al., 2004).

Animal miRNAs are identified as part of an 80-nt RNA with stem-loop structures (pre-miRNA) that are included in several hundred or thousands of nucleotide-long miRNA precursor, named primary miRNA precursor (pri-miRNA) (Fig. 1). Until now, a few different pri-miRNA precursors have been isolated and characterized, three from human, one from C. elegans, and one from plants. They are all capped, polyadenylated, and apparently noncoding: the human cluster miR-23a–27a–24-2 primary precursor is an unspliced ~2.2 kb RNA; in contrast, the pri-miRNA for human miR-155 (BIC) includes two introns and two alternative polyadenylation sites capable of producing two spliced pri-miRNAs of 0.6 and 1.4 kb (Cullen, 2004).

The production of miRNAs from pri-miRNA is a complex and coordinated process where different groups of enzymes and associated proteins, located in the nucleus or cytoplasm, operate the multistep maturation of these tiny RNAs. Principally, the maturation process of miRNAs can be resumed in three important steps: cropping, export, and dicing.

In the cropping step, the pri-miRNA is converted to pre-miRNA through the cleavage activity of the Drosha enzyme, a nuclear Ribonuclease III endonuclease capable of cropping the flank regions of pri-miRNA, in turn to liberate the 60–70-nt pre-miRNA (Bartel, 2004). Different structural requisites are needed to achieve efficient precursor maturation by Drosha: first, a large terminal loop (>10 nt) in the hairpin and a stem region one turn bigger than the pre-miRNA; second, 5’ and 3’ single-stranded RNA (ssRNA) extensions at the base of the future miRNAs (for review see Filipowicz et al., 2005; Tomari and Zamore, 2005). It has been proposed that Drosha may recognize the primary precursor through the stem-loop structure, and then cleave the stem at a fixed distance from the loop to liberate the pre-miRNA. How the enzyme is capable of discriminating the pri-miRNA stem-loop structure with respect to the stem-loops of other cellular RNAs is not clear, but probably proteins associated with Drosha confer specificity to this process. In fact, Drosha has been found as a part of a large protein complex of ~650 kDa, which is known as the “Microprocessor,” where Drosha interacts with its cofactor, the Di George syndrome critical region gene 8 (DGCR8) protein in human and Pasha in Drosophila melanogaster (Landthaler et al., 2004). The Microprocessor appears to represent a heterotetramer consisting of two Drosha and two DGCR8 molecules; because DGCR8 contains two consensus dsRNA binding domains, this protein may play an important role in substrate discrimination and binding.

The resulting product of cropping, the pre-miRNA, presents a 5’ phosphate and 3’ hydroxyl termini, and two or three nucleotides with single-stranded overhanging ends, classic characteristics of RNase III cleavage of dsRNAs. After the Microprocessor nuclear activity, the produced pre-miRNA is exported to the cytoplasm by Exportin-5 (Exp5)/RanGTP (Kim, 2004). Exp5 forms a nuclear heterotrimer with RanGTP and the pre-miRNA from Drosha processing. This interaction, which is dependent on RNA structure but independent of sequence, stabilizes the nuclear pre-miRNA and promotes the export to the cytoplasm. In the export step, once the Exp5-RanGTP-pre-miRNA complex has reached the cytoplasm through the nuclear pore, the
RanGTP is hydrolyzed to RanGDP and the pre-miRNA is released. Following arrival into the cytoplasm, the pre-miRNA is processed into 18–22-nucleotide miR duplexes by the cytoplasmic RNase III Dicer and, in humans, its partner TRBP. The PAZ domain of Dicer is thought to interact with the 3' overhang nucleotides present in the pre-miRNA hairpin, while the dsRNA binding domain binds the stem and defines the distance of cleavage from the base of pre-miRNA. The cleavage products of 22-nt-long miRNA duplexes have a reduced half-life. Normally, one strand of this duplex is degraded, whereas the other strand accumulates as a mature miRNA. Studies on siRNAs have highlighted that the selection of the right strand is related to the thermodynamic stability of the duplex, and the strand with relatively unstable base pairs at the 5' end usually represents the mature miR.

**MiRNA in Action: RISC and Gene Target Inhibition**

In the RNA duplex produced from the Dicer activity, the mature miRNA is only partially paired to the miRNA*, the small RNA that resides on the opposite pre-miRNA stem. From the miRNA-miRNA* duplex, only the miRNA enters preferentially in the protein effector complex, the RNA-induced silencing complex (RISC) or miRgonaute, which mediates the degradation or translation inhibition of mRNAs target gene (Tang, 2005).

Animal miRNAs are imperfectly paired to the 3' UTR of target mRNA and inhibit the protein production by an unknown and very controversial mechanism; in some cases, the miRNAs show nearly precise complementarity to their target and trigger mRNA degradation as siRNA in the RNA interference process (Tang, 2005).

Several proteins have been identified as essential components of RISC, but only a few have been functionally characterized in the posttranslational regulation. The core components of RISC are members of the Argonaute (Ago) protein family, whose members present a central PAZ domain like Dicer and a carboxy terminal PIWI domain. This domain binds the miR/miR* duplex to the 5' end, whereas the PAZ domain binds to the 3' end of ssRNAs; moreover, structural and biochemical studies have suggested that the Ago proteins are the target-cleaving endonucleases of RISC, and in this activity the complex is helped and coordinated by other proteins whose functions are...
not really understood, such as RNA-binding protein VIG, the Fragile-X related protein in Drosophila, the exonuclease Tudor-SN, and many other putative helicases (Nelson et al., 2003).

In human cells, after transfection of miRNAs by vectors or miRNA precursors, and subsequent activation of RISC activity, the core component of RISC, together with the triggering miRNA target mRNA, is concentrated in cytoplasmic foci known as Processing bodies (P-bodies) or GW-bodies. In accordance with this triggered RISC localization, the researchers thought that the miRNAs, in association with AGO proteins, might be capable of repressing the translation at the ribosomal level and to relocalize the mRNA targets to the P-bodies (Liu et al., 2005).

The most important characterization of the function of miRNAs is the identification of the mRNA targets. Because animal miRNAs have a 5′end-restricted complementarity to the mRNA target (only five to eight nucleotides of perfect complementarity, an RNA sequence named "seed region"), the miRNAs are predicted to regulate a large number of animal genes. Different algorithms have been developed to predict the animal miRNA targets; they are based on different criteria, resulting from the analysis of targets demonstrated in vivo: 1) perfect or nearly perfect base-pairing at the seed region and thermodynamic stability of the duplex miRNA-mRNA; 2) phylogenetic conservation of the seed region; 3) multiple target sites in a single target by the same or different miRNAs; and 4) absence of strong secondary structures at the miR-binding site of the target. Several computational procedures are available to predict miRNA targets, such as Diana-MicroT (http://www.diana.pcbi.upenn.edu/cgi-bin/micro_t.cgi), TargetScan (http://genes.mit.edu/targetscan/), and miRanda (http://www.microrna.org/miranda_new.html) (Enright et al., 2003; Kiriakidou et al., 2004; Lewis et al., 2005).

### Table 1. Examples of functions of miRNAs in animals.

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Species</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>lin-4</td>
<td>C. elegans</td>
<td>Regulation of life span (proportionally with expression)</td>
<td>(Boehm and Slack, 2005)</td>
</tr>
<tr>
<td>Mir-273</td>
<td>C. elegans</td>
<td>Controls laterality of the chemosensory system</td>
<td>(Chang et al., 2004)</td>
</tr>
<tr>
<td>Mir-1</td>
<td>Drosophila</td>
<td>Control of muscle during larval growth</td>
<td>(Sokol and Ambros, 2005)</td>
</tr>
<tr>
<td>Mir-2, mir-6, mir-11, mir-13, mir-308</td>
<td>Human, Drosophila</td>
<td>Regulate/suppress embryonic apoptosis by posttranscriptional repression of proapoptotic factors</td>
<td>(Zhao et al., 2005)</td>
</tr>
<tr>
<td>Mir-2, mir-13</td>
<td>Drosophila</td>
<td>Normal development of head and posterior abdominal segments</td>
<td>(Boutla et al., 2003)</td>
</tr>
<tr>
<td>Mir-7</td>
<td>Drosophila</td>
<td>Promotes photoreceptor differentiation</td>
<td>(Li and Carthew, 2005)</td>
</tr>
<tr>
<td>Mir-14</td>
<td>Drosophila</td>
<td>Suppression of cell death; normal fat metabolism</td>
<td>(Xu et al., 2003)</td>
</tr>
<tr>
<td>Mir-278</td>
<td>Drosophila</td>
<td>Control of energy homeostasis by influencing insulin production</td>
<td>(Teleman and Cohen, 2006)</td>
</tr>
<tr>
<td>bantam</td>
<td>Drosophila</td>
<td>Controls cell proliferation and prevents apoptosis</td>
<td>(Brennecke et al., 2003)</td>
</tr>
<tr>
<td>Mir-1-1</td>
<td>Mouse</td>
<td>Control of balance between differentiation and proliferation during cardiogenesis</td>
<td>(Zhao et al., 2005)</td>
</tr>
<tr>
<td>Mir-122</td>
<td>Mouse</td>
<td>Regulator of cholesterol and fatty-acid metabolism</td>
<td>(Esau et al., 2006)</td>
</tr>
<tr>
<td>miR-142s</td>
<td>Mouse</td>
<td>B lymphocyte differentiation; Myeloid differentiation</td>
<td>(Chen et al., 2004)</td>
</tr>
<tr>
<td>Mir-181</td>
<td>Mouse</td>
<td>B lymphocyte differentiation; Myeloblast differentiation by targeting Hox-11</td>
<td>(Chen et al., 2004)</td>
</tr>
<tr>
<td>Mir-196</td>
<td>Mouse</td>
<td>Limb development, acting upstream of Hoxb8 and Sonic hedgehog</td>
<td>(Hornstein et al., 2005)</td>
</tr>
<tr>
<td>miR-200a, miR-141, miR-429, miR-199a</td>
<td>Mouse</td>
<td>Skin morphogenesis</td>
<td>(Yi et al., 2006)</td>
</tr>
<tr>
<td>Mir-223</td>
<td>Mouse</td>
<td>Myeloid differentiation</td>
<td>(Chen et al., 2004)</td>
</tr>
<tr>
<td>Mir-15a, mir-16-1</td>
<td>Human</td>
<td>Regulation of granulopoiesis</td>
<td>(Garzon et al., 2006)</td>
</tr>
<tr>
<td>Mir-375</td>
<td>Human</td>
<td>Regulation of insulin secretion</td>
<td>(Poy et al., 2004)</td>
</tr>
</tbody>
</table>

*Birth Defects Research (Part C) 78:180–189, (2006)*
miRNAs: FUNCTIONS IN NORMAL AND DISEASE STATES

With the discovery of new members of the miRNA family on a daily basis, it becomes evident that these small genes must be involved in normal cellular homeostasis (Ambros, 2004; Bartel, 2004; Sevignani et al., 2006). Furthermore, with the development of new techniques for genome-wide screening of miRNA expression, abnormal levels of miRNAs were identified in malignant cells with respect with normal counterparts (Esquela-Kerscher and Slack, 2006; Hammond, 2006; Calin and Croce, 2006). The functions of miRNAs, initially a “shadow” area of research, revealed a general participation in every functional aspect of normal cells in organisms with different degrees of complexity. For example, in Drosophila, miR-14 suppresses cell death and is required for normal fat metabolism (Xu et al., 2003), while bantam encodes a developmentally regulated miRNA that controls cell proliferation and regulates the pro-apoptotic gene hid (Brennecke et al., 2003). As shown in Table 1, participation of miRNAs in essential biological processes has been consistently proven, such as cell proliferation control (miR-125b and let-7), hematopoietic B-cell lineage fate (miR-181), B-cell survival (miR-15a and miR-16-1), brain patterning (miR-430), pancreatic cell insulin secretion (miR-375), and adipocyte development (miR-143) (for reviews see Harfe, 2005; Miska, 2005; Hwang and Mendell, 2006).

As a consequence of extensive participation in normal functions, it is quite logical to ask the question if abnormalities in miRNAs should have importance in human diseases. The answer to this fundamental question is built on many recent investigations, obtained mainly from the study of human cancers. As shown in Table 2, miRNAs and/or proteins involved in the processing of miRNAs are involved in various types of human diseases. miRNAs can act both as tumor suppressors and oncogenes (Esquela-Kerscher and Slack, 2006; Hammond, 2006; Calin and Croce, 2006). Homozygous deletions (as is the case for miR-15a/miR-16a

![Table 2. Examples of miRNAs involvement in human diseases.](image-url)
Figure 2. miRNAs as cancer players: mechanism of alterations. For details, see text pages 185 and 187. Modified with permission from Calin et al. (2004b).
cluster), and the combination mutation plus promoter hypermethylation or gene amplification (as is the case of miR-155 or the cluster miR-17–92) appear to be the main mechanisms of inactivation or activation, respectively (He et al., 2005b; Lu et al., 2005; O'Donnell et al., 2005). Because of the small size of miRNAs, loss-of-function or gain-of-function point mutations represent rare events (Calin et al., 2005a). miRNAs activity can be influenced either by the reposition of other genes close to promoters/regulatory regions of miRNAs (as is the case of mir-142s – c-MYC translocation), or by the relocalization of an miRNA near other regulatory elements. The overall effects in the case of miRNA inactivation is the overexpression of target mRNAs, while miRNA activation will lead to downregulation of target mRNAs involved in apoptosis, cell cycle, invasion, or angiogenesis (Fig. 2).

To date, only a few miRNA::mRNA interactions with importance for cancer pathogenesis have been proven (Calin et al., 2005b; Calin and Croce, 2006). It was elegantly demonstrated that the let-7 miRNA family regulates RAS oncogenes and that let-7 expression is lower in lung tumors than in normal lung tissue, while RAS protein has an inverse variation (Johnson et al., 2005). Furthermore, enforced expression of the miR-17–92 cluster from chromosome 13q32–33, in conjunction with c-myc, accelerates tumor development in a mouse B-cell lymphoma model (He et al., 2005b). Two miRNAs from the same cluster, miR-17–5p and miR-20a, negatively regulate the E2F1 transcription factor, a gene shown to function as a tumor suppressor in some experimental systems (O'Donnell et al., 2005).

Recently, an unexpected mechanism of miRNAs involvement in human disease was identified. Tourette’s syndrome (TS) is a neurologic disorder manifested particularly by motor and vocal tics, and is associated with behavioral abnormalities. Sequence variants of a candidate gene on chromosome 13q31.1 named SLITRK1 (Slit and Trk-like 1) were identified in patients with TS. One of them, named var321, found in two unrelated patients, was located in the 3'UTR binding site for the miR-189 and might affect SLITRK1 expression (Abelson et al., 2006). This mechanism of abnormal miRNA::miRNA interaction seems to be a general one, as it was shown also in the case of oncogene c-KIT. Three of the highly overexpressed miRNAs in thyroid cancers, miR-221, miR-222, and miR-146, are predicted to interact with the KIT oncogene mRNA at two different sites. Tumors in which the upregulation of these miRNAs was the strongest showed dramatic loss of KIT, and in half of the cases the downregulation was associated with germline single nucleotide polymorphisms (SNP) in the two recognition sites in KIT for these three miRNAs (He et al., 2005a).

One important proof for the functional importance of such abnormalities is represented by the reproduction of similar diseases in mouse models with abnormal miRNA expression. Recently, the first example of a transgenic miRNA mouse was published: as expected by the overexpression of miR-155 in human lymphomas (Eis et al., 2005; Jiang et al., 2006), the miR-155 transgenic mice overexpressing the gene only in B cells, exhibit a preleukemic pre-B cell proliferation in spleen and bone marrow, followed by frank B cell malignancy (Costinean et al., 2006). These findings indicate that the role of miR-155 is to induce polyclonal expansion, favoring the capture of secondary genetic changes for full transformation. This is an exciting proof that deciphering miRNA alterations is important, and that miRNAs, as small as they are, represent big culprits in human diseases.

ACKNOWLEDGMENTS

We thank Cinzia Sevignani for the assistance with figures and Dimitri Iliopoulos for the helpful comments and discussions. We apologize to many colleagues whose work was not cited due to space limitations.

REFERENCES


Takamizawa J, Konishi H, Yanagisawa K, et al. 2004. Reduced expression of
the let-7 miRNAs in human lung cancers in association with shortened
Tam W. 2001. Identification and char-
acterization of human BIC, a gene on
chromosome 21 that encodes a non-
Tang G. 2005. siRNA and miRNA: an in-
sight into RISCs. Trends Biochem Sci
30:106–114.
Teleman AA, Cohen SM. 2006. Drosophila lacking miRNA miR-278 are defec-
tive in energy homeostasis. Genes Dev
15:417–422.
Tomari Y, Zamore PD. 2005. miRNA bi-
genesis: drosha can’t cut it without a
Wightman B, Ha I, Ruvkun G. 1993. Post-
transcriptional regulation of the het-
erochronic gene lin-14 by lin-4 mediates
temporal pattern formation in C. el-
The Drosophila miRNA Mir-14 sup-
presses cell death and is required for
normal fat metabolism. Curr Biol 13:
790–795.
Yanaihara N, Caplen N, Bowman E, et al.
2006. miRNA signature in lung cancer
diagnosis and prognosis. Cancer Cell
Morphogenesis in skin is governed by
discrete sets of differentially expressed
Serum response factor regulates a mus-
cle-specific miRNA that targets Hand2
220.