

# MicroRNAs: molecular features and role in cancer

Elodie Lages<sup>1</sup>, Hélène Ipas<sup>1</sup>, Audrey Guttin<sup>1</sup>, Houssam Nesr<sup>1</sup>, François Berger<sup>1</sup>, Jean-Paul Issartel<sup>1,2\*</sup>

<sup>1</sup> GIN, Grenoble Institut des Neurosciences INSERM : U836, CEA, Université Joseph Fourier - Grenoble I, CHU Grenoble, UJF - Site Santé La Tronche BP 170 38042 Grenoble Cedex 9,FR

<sup>2</sup> Institut de Biologie et Pathologie CHU Grenoble, Grenoble,FR

\* Correspondence should be addressed to: Jean-Paul Issartel <jean-paul.issartel@ujf-grenoble.fr >

## Abstract

microRNAs (miRNAs) are small noncoding endogenously produced RNAs that play key roles in controlling the expression of many cellular proteins. Once they are recruited and incorporated into a ribonucleoprotein complex miRISC, they can target specific mRNAs in a miRNA sequence-dependent process and interfere in the translation into proteins of the targeted mRNAs via several mechanisms. Consequently, miRNAs can regulate many cellular pathways and processes. Dysregulation of their physiological roles may largely contribute to disease. In particular, in cancer, miRNAs can be involved in the deregulation of the expression of important genes that play key roles in tumorigenesis, tumor development, and angiogenesis and have oncogenic or tumor suppressor roles. This review focuses on the biogenesis and maturation of miRNAs, their mechanisms of gene regulation, and the way their expression is deregulated in cancer. The involvement of miRNAs in several oncogenic pathways such as angiogenesis and apoptosis, and in the inter-cellular dialog mediated by miRNA-loaded exosomes as well as the development of new therapeutical strategies based on miRNAs will be discussed.

**MESH Keywords** Animals ; Apoptosis ; Cell Proliferation ; Gene Expression Regulation ; Gene Rearrangement ; Humans ; MicroRNAs ; genetics ; metabolism ; therapeutic use ; Models, Biological ; Mutation ; Neoplasms ; etiology ; genetics ; metabolism ; pathology ; RNA, Messenger ; genetics ; metabolism ; RNA, Neoplasm ; genetics ; metabolism ; RNA-Induced Silencing Complex ; genetics ; metabolism ; Tumor Markers, Biological ; genetics ; metabolism

## Introduction

miRNAs were described in *Caenorhabditis elegans* at the beginning of the 1990s (1) as small noncoding endogenously produced RNAs that inhibit mRNA translation. Nevertheless, the existence in eukaryotic cells of RNA molecules called tcRNAs (translational control RNAs), that were able to inhibit the translation of mRNAs, had been previously suggested by Heywood *et al.* (2). The first miRNA described in *C. elegans*, lin-4, is a small RNA containing complementary sequences for repeated elements of the 3'UTR region of the lin-14 and lin-28 mRNAs. Lee *et al.* proposed a lin-4-dependent regulation of the translation of the lin-14 and lin-28 mRNAs due to a sense-antisense RNA interaction. This regulation model was validated by Wightman *et al.* who used reporter genes and demonstrated that the 3' untranslated region (UTR) of lin-14 mRNA was essential for the regulation of its translation by lin-4 (3). A second small RNA, let-7, which, similar to lin-4, comes from the family of heterochronic switch genes, and controls the larval development of *C. elegans* was then discovered by Reinhart *et al.* (4). let-7 plays a role in the transition of the larval L3/L4 stage to the adult stage, repressing the expression of LIN-41 by interacting with the 3'UTR region of the mRNA.

lin-4 and let-7 were first called small temporal RNAs (stRNAs) (5) due to their role in the control of development and were renamed miRNAs in 2001 (6) (Figure 1). Afterwards, several miRNAs were described in other organisms such as *Drosophila melanogaster*, mice, and plants as well as in human cell lines (7–13).

## miRNA: nomenclature, biogenesis and functions

### miRNA nomenclature

miRNAs are identified either by cloning and sequencing studies or informatics approaches using prediction programs based on conservation of miRNA within species and on the detection of the stem-loop structures typical of miRNA precursors. All miRNAs named in scientific publications and generally found using cloning and sequencing techniques are listed in the database called miRBase (<http://microrna.sanger.ac.uk/> (14)). miRBase version 18 (November 2011) now classifies 1527 miRNAs for humans.

miRNA-encoding genes are first transcribed in stem-loop structured primary transcripts called pri-miRNAs, which are then excised by RNase III Drosha and its cofactor DGCR8, freeing a precursor called pre-miRNA. These precursor molecules are then exported from the nucleus to the cytoplasm with exportin-5. They are then matured by the RNase III Dicer to free a duplex miRNA-miRNA\*. One strand, called mature, is then incorporated into the RISC complex, a complex containing essentially Argonaute-type proteins. (Figure 2).

While genes or pre-miRNAs are noted "mir -X", mature miRNAs are noted "miR -X" where X is a numeric value. A three-letter code is added as a prefix to refer to the organism they originate from. One letter stands for the organism genus and the two others for the species, e.g., *hsa* for *Homo sapiens* or *mmu* for *Mus musculus*.

When mature miRNAs differ by a few bases, a letter is added as a suffix, reflecting the difference: *e.g.* , miR-181a and miR-181b:

- miR-181a: aacaucaacgcugucggugagu
- miR-181b: aacaucauugcugucgggggu

When both strands of a pre-miRNA are processed as mature miRNAs, an indication is given to specify which arm of the hairpin structure of the pre-miRNA generates one or the other of the two miRNAs. Several nomenclatures can be used:

- miR-X and miR-X\*: miR-X\* is the less abundant of the two mature miRNAs in the cells.
- miR-X-5p and miR-X-3p: 5p and 3p stand for the 5' and 3' arms of the stem-loop structure of the pre-miRNA, respectively. This nomenclature is mainly used when the respective abundance of the two miRNAs is still unknown (15).
- miR-X-s (5' arm) and miR-X-as (3' arm).

Moreover, a mature miRNA can result from the transcription and maturation of transcripts from separate genomic loci. A numeral suffix is added to the names of all the multiloci miRNAs, *e.g.* , miR-181a-1 and miR-181a-2 (miR-181a-1 originates from a locus on chromosome 1 and miR-181a-2 from a locus on chromosome 9). Nevertheless, some miRNAs, such as hsa-let-7 and cel-lin-4, do not follow the above-described nomenclature.

### **Interfering RNAs: similarities and differences**

RNA interference (RNAi) is mainly responsible for the post-transcriptional regulation of gene expression (mRNA cleavage or decay, translation repression or activation) but is also involved in transcriptional regulation as described by various studies (16–19).

Mainly two classes of small RNAs interfere in the RNA interference process: miRNAs and siRNAs (short interfering RNA). These RNAs share similarities such as their short size (about 22 nucleotides), their maturation by the ribonuclease III Dicer, and their implication in the RISC complex for post-transcriptional repression.

Nevertheless, there are a number of important differences, such as the following:

- miRNAs are exclusively endogenously produced. They come from intrinsic genes of the organism they interfere in, whereas siRNAs may originate from viruses, transposable elements, perfectly paired endogenous double-stranded RNA resulting from an antisense transcription of various loci (20), or transfected exogenous and double-stranded RNAs.
- miRNAs play a role in the regulation of genes that do not generate them, whereas siRNAs mainly regulate the genes that produce them. This point is critical in order to secure host cell defense against parasite RNAs such as viruses or transposons.
- In mammals, miRNAs have a varying degree of complementarity with their target, whereas siRNA always have a perfect complementarity with their targets. When the complementarity is perfect, the target mRNA is degraded in the presence of the Ago2 protein, which is the sole protein of the four Argonaute family proteins responsible for endonucleolytic cleavage. In plants, the difference between miRNAs and siRNAs is very small because the pairing between miRNA and mRNA is often perfect (21).

In addition to miRNAs and siRNAs, there are other small RNAs that contribute to maintaining the genome integrity: the piRNAs (piwi-interacting RNAs). They are longer (26–31 nucleotides), abundant in germinal cells but can also be found in somatic tissues (22–24) (Figure 3). These RNAs, formerly called repeat-associated siRNA (rasiRNA), were discovered in *D. melanogaster* (25). In 2006, several studies showed the presence of piRNAs in mammals (25–28). Contrary to siRNA and miRNA, it seems that they come from long single-stranded RNA precursors and their biogenesis does not include the RNase III Dicer. Once matured, piRNAs associate themselves with PIWI proteins, a subfamily of Argonaute proteins, principally to target transposons and preserve genome integrity (22).

### **miRNA biogenesis**

#### ***Localization and structure of miRNA coding genes***

These genes are widespread on all chromosomes except for chromosome Y and approximately 50% of the described miRNAs are located in clusters. When these clustered miRNAs are under the control of a single promoter, they are dubbed polycistronic miRNAs.

miRNA coding genes can be independent gene units and miRNAs are then called intergenic; they can also be gene units coding for pre-mRNA that either code for proteins or do not code for them. In this case, miRNAs can be intronic or exonic (Figure 4) (29–31).

#### ***Transcription of primary miRNA transcript***

Most of the miRNA coding genes are transcribed by the RNA polymerase II, producing long primary transcripts (pri-miRNAs). These pri-miRNAs have a typical structure composed of a stem (about 33 nucleotides), a loop connecting the two strands of the stem, and single-stranded flanking sequences (Figure 5). Like mRNAs, these sequences contain a 7-methyl-guanosine (m7G) cap at their 5' end and a 3' polyA tail (32, 33). With the transcription performed by the RNA polymerase II, the regulation of this mechanism could be similar to the regulation of the transcription of protein-coding genes. The localization of promoter regions of miRNA coding genes was predicted *in silico* by genomic mapping of the transcription start sites (TSS) and by mapping of transcription factor binding motifs. This was reported in *Arabidopsis thaliana*, *C. elegans*, and *H. sapiens* (34–36). Furthermore, two studies highlighted the localization and characteristics of TSS sequences of miRNAs in human using high-throughput genomic analyses such as chromatin immunoprecipitation (37, 38).

### **Maturation of pri-miRNAs into miRNAs**

#### **Formation of the pre-miRNA in the nucleus**

In human, primary transcripts are processed in the nucleus by a protein complex called microprocessor that is composed of the ribonuclease Drosha associated with its cofactor DGCR8 (DiGeorge syndrome Critical Region gene 8) (39). The Drosha complex also contains several other factors such as EWSR1, FUS, numerous heterogeneous nuclear ribonucleoproteins (hnRNPs), p68 (DDX5) and p72 (DDX17) DEAD-box helicases. The roles of these accessory components in miRNA maturation is largely unknown, some hnRNPs and p68/p72 may promote the fidelity and activity of the Drosha processing (40, 41). DGCR8, which interacts with double-stranded RNA, is required to allow the binding of the Drosha/DGCR8 complex to the pri-miRNA at the junction between the flanking sequences and the stem-loop. Once the complex is bound to the pri-miRNA, DGCR8 specifies the Drosha cleavage distance (~11 bp) away from the junction between the flanking sequences and the stem-loop. The Drosha enzyme is a class 2 ribonuclease III, *i.e.*, it contains two RNase III domains and one binding domain for double-stranded RNA. This structure allows an asymmetric cleavage of the double-stranded RNA and the release of a shortened pri-miRNA stem-loop (42). This shortened stem-loop is called pre-miRNA and contains two unpaired nucleotides at the 3' end and a phosphate group at the 5' end (43) (Figure 5).

Another pre-miRNA biogenesis pathway is the mirtron pathway, which is connected to the mRNA splicing mechanism. After splicing and lariat release, the intron structures itself in a stem-loop to generate a pre-miRNA. In this case, the mechanism of mirtron maturation bypasses cleavage by the pre-miRNA generating enzyme Drosha. The pre-miRNA 5' and 3' ends correspond to the donor splicing site (GU) and the acceptor splicing site (AG), respectively. This mirtron pathway, first described in the invertebrates *D. melanogaster* et *C. elegans* (44, 45), was then evidenced in mammals (such as miR-877 and miR-1224) (46). The main difference between the invertebrate and mammal mirtron pathways seems to rely on the fact that the mature miRNA is usually located on the 3' strand of the stem-loop in invertebrates while it is located on the 5' strand in mammals (Figure 6).

#### **Pre-miRNAs export from the nucleus to the cytoplasm**

The export of RNAs (messenger, ribosomal, and transfer RNAs) is made possible by the action of receptors of the karyopherin family, such as CRM1 and exportin-t (Exp-t). Nevertheless, nuclear-export of pre-miRNAs mostly relies on another receptor called exportin-5 (Exp-5) (47, 48). This receptor, discovered in 2002, belongs to the karyopherin  $\beta$  family. Like all the other receptors of this family, it binds directly and specifically to the RAN-GTP protein (RAS-related nuclear protein with bound GTP) in the nucleus and then binds to nucleoporins to allow the pre-miRNA transfer from the nucleus to the cytoplasm (49). Exp-5 specifically interacts with double-stranded RNAs longer than 14 bp that contain a protruding 3' end (50), hence its binding specificity for pre-miRNAs (51). Once the pre-miRNA/Exp-5/RAN-GTP complex is assembled and is bound to nucleoporins, it goes through the nuclear pores. Then the RAN-GAP enzyme (RAN-GTPase activating protein), located on the cytoplasmic side of the pores, activates the hydrolysis of the RAN-bound GTP into RAN-bound GDP (52), leading to the release of the pre-miRNA in the cytoplasm (Figure 7).

#### **Mature miRNA production in the cytoplasm**

In the cytoplasm, pre-miRNAs become the substrates of the ribonuclease III Dicer, which completes their processing. Previously described for siRNA processing (53), Dicer was found to be involved in the final cleavage of pre-miRNAs into 21-nucleotide miRNAs in *C. elegans* (54, 55). Dicer is a class 3 type III ribonuclease composed of two RNase III domains, one double-stranded RNA (dsRNA) binding domain, one PAZ signature (Piwi/Argonaute/Zwille), and a helicase domain. The PAZ domain allows Dicer to recognize the single-stranded 3' overhangs of the pre-miRNA (56, 57). In addition, the dsRNA binding domain allows the recognition of the stem by Dicer (58, 59) and the cleavage by the RNase III domains of the dsRNA about 21 nt away from the PAZ-bound end. This cleavage generates another single-stranded 3' overhang (Figure 8).

### **Gene expression regulation by miRNAs**

#### **miRISC (miRNA-induced silencing complex) formation**

During the maturation step catalyzed by Dicer, two other proteins, TRBP (TAR (HIV-1) RNA binding protein or TARBP2) and Ago2 (Argonaute 2), were found to be involved in the formation of the RISC loading complex (RLC) with Dicer (60–63). Most of the studies

carried out *in vitro* with recombinant proteins revealed that the association of only three proteins – Dicer, TRBP, and Ago2 – makes an equistoichiometric complex that is able to process the pre-miRNA and to select one of the two strands to form the miRISC. The dimeric TRBP (64) first dissociates into monomers to form the RLC. In *Drosophila*, the assembly of the Dicer/TRBP/Ago complex requires ATP (65), whereas in human, assembly of the complex can be either ATP-independent (61–63) or ATP-dependent (66).

The RNA duplex obtained after maturation contains 2-nt 3' overhang ends, resulting from the action of the two type III RNases, Drosha and Dicer. In some rare cases, the two duplex strands can accumulate in the cell (6, 67) with both strands acting as mature strands to guide the miRISCs to the mRNA targets (68). Nevertheless, most of the time only one of the two strands is selected according to asymmetric rules (69, 70). The unselected strand is called the passenger strand and noted miR-X\* (see nomenclature Part 3.1). The selected strand is called the guide strand or mature strand and noted miR-X. This strand has the less thermodynamically stable 5' end, *i.e.*, with the weakest 4-bp-long duplex binding energy (70). Indeed, this thermodynamic instability can facilitate the unwinding of the duplex by helicases, such as Gemin3 (67) or RCK/p54 (71). Unwinding of the duplex may also be promoted by Dicer's helicase domain, since it was shown that it can be performed by the *in vitro* associated RLC (only containing Dicer/TRBP/Ago2) (63). ATP does not seem to be necessary for the dissociation of the two strands by a helicase. Indeed, the energy released after the cleavage of the two phosphodiester bonds by Dicer could provide energy to achieve this dissociation step (63). Another recent study suggests that Ago1 and Ago2 may participate in the separation of the duplex's two strands (72). For siRNA duplexes, after the dissociation of the two strands, the passenger strand is degraded by cleavage by Argonaute proteins (73, 74). As for the miRNAs duplexes, the fate of the passenger strand seems less clearly identified. According to Matranga *et al.*, degradation by cleavage does not occur because of the imperfect complementarity between the two strands (75). According to Shin *et al.*, the passenger strand of a miRNA duplex may be degraded if strong complementarity is observed in the middle part of the duplex (76). Another pathway has also been proposed by Diederichs and Haber, who claim that the passenger strand is cleaved by Ago2 before Dicer's action (77).

RLC allows *in fine* the loading of the mature miRNA into the miRISC after the dissociation of the two strands. Among the three proteins that take part in the RLC (Dicer, TRBP, and Ago), only the Ago component is required for RISC activity in the miRISC (Figure 9). Argonaute proteins are composed of two main regions: one containing the N-terminal and the PAZ domains, the other one containing the Mid and PIWI domains (78). Although the functions of these domains are poorly defined, it seems that the three domains – PAZ, Mid, and PIWI – play an important role in the RNA interference phenomenon as they are required for the interaction with the miRNA. Indeed, the PAZ domain, which is similar to the Dicer domain, binds to RNA duplexes containing two unpaired nucleotides on the 3' end and therefore recognizes the 3' end of mature miRNAs (56, 57, 79). The Mid domain recognizes the phosphate 5' end of mature miRNAs and also mRNA m7G capping (80, 81). Finally, the PIWI domain contains a tertiary structure specific of the RNase H family providing an endonucleolytic activity to RISC (82). In *Drosophila*, only Ago1 is specialized in the loading of miRNAs (Ago2 is specialized in loading siRNAs) (83). In human, there are four Argonaute proteins (Ago1–4). All four forms can load miRNAs, but only Ago2 has an endonucleolytic activity (84, 85).

### ***Interaction between miRNAs and mRNA targets***

#### ***Mechanisms of interaction***

The main interaction between miRNAs and target messenger RNAs occurs at the level of the mRNA 3'UTR and generates duplexes. In plants, most miRNAs show a perfect complementarity with their mRNA targets and perfect duplex formation is associated with the degradation of the targets by endonucleolytic cleavage (86, 87). On the contrary, in metazoans, a perfect complementarity between miRNAs and their mRNA targets is not always the rule.

Several specific features of duplex formation have been described which were used to define algorithms to predict miRNA targets. In 2003, Lewis *et al.* reported that the hybridization between miRNA and its mRNA target has to be perfectly complementary between nucleotides 2 and 7 from the 5' end of the miRNA; this region was called the seed region (88). Any mismatch in this region and in particular any G:U type mismatch would more or less severely reduce the miRNA efficiency to interact with a given mRNA target, depending on the size of the seed region (89, 90). For example, with a 6-nt seed region, a G:U type mismatch leads to a complete loss of the miRNA activity. In addition, the presence of an A residue in position 1 and/or an A/U residue in position 9 from the 5' end of the miRNA (*i.e.*, on both sides of the seed region) may improve the interaction between miRNA and the targeted mRNA (91).

Another feature is the presence of a mismatched bulge in the middle region of the miRNA–mRNA duplex, which prohibits the endonucleolytic cleavage catalyzed by Ago2 (92). In addition, a sequence complementarity on the 3' end of the miRNA stabilizes the miRNA–mRNA duplex, especially when it occurs between nucleotides 13 and 16 from the 5' end of the miRNA (93).

Finally, other factors can improve the miRNA efficiency such as AU-repeat mRNA sequences close to the miRNA binding site, or the proximity of the miRNA site to the stop codon or to the polyA tail of the target, especially for mRNA with long 3'UTR (>1300 nt). However, for a distance between the miRNA site and the stop codon shorter than 15 nucleotides a severe drop in miRNA efficiency is expected. Proximity to the stop codon and to the polyA tail of the miRNA site increases the accessibility of the miRISC. Moreover, the

binding of several different miRNAs to the 3'UTR promotes the cooperative repressing phenomenon (94). All these structural features are summarized in Figure 10.

Besides the above-mentioned canonical mechanisms of miRNA gene regulation through 3'UTR interactions, other "non-canonical" miRNA-mediated mechanisms of mRNA expression modulation have been reported. Some miRNAs have been shown to bind to the open reading frame (95) or to the 5'UTR of the target genes (96).

### ***Categories of target sites***

Several categories of target sites have been described according to their structural features (91, 97). There are two main categories, which can be called canonical or marginal sites, and more rarely, atypical sites (Figure 11). Canonical sites are characterized by strong pairing in the seed region and contain three types of sites (8mer, 7mer-m8, and 7mer-A1). The 8mer sites have an interaction on 8 nucleotides including the 6 seed region nucleotides (nucleotides 2–7), and nucleotides in positions 1 and 8. The 7mer-m8 and 7mer-A1 sites include the 6 seed region nucleotides and, respectively, any nucleotide in position 8 (m8) or the A residue in position 1. Marginal sites are characterized by a weaker pairing in the 5' end of the miRNA, with pairing of only 6 nucleotides located inside the seed region (6mer) or located between nucleotides 3 and 8 (6mer offset). Finally, atypical sites are characterized by an increased number of complementary nucleotides at the 3' end.

### ***Biocomputational tools for the prediction of target sites***

Several tools and databases have been set up to assist in the identification of the mRNAs that are putatively targeted by miRNAs in mammals. Target identification is based on different criteria such as the binding duplex stringency characterized by the length of the pairing region and the thermodynamic stability of this binding, the conservation of the target sites between species, or the number of miRNAs targeting a specific 3'UTR part. The main biocomputational tools are referenced in Table 1.

TargetScanS is considered the simplified and improved version of TargetScan. Indeed, in this version, the necessary pairing is only 6 nucleotides in the seed region and the target site number on a single mRNA is not required in this version. The three biocomputational tools, TargetScanS, PicTar, and EIMMo have many common putative target sites because the engines look for sites with strong base-pairing in the seed region. However, there is no perfect overlapping between the results obtained with these three engines that use different UTR databases and/or miRNA sequence files (105). Moreover, these algorithms have some intrinsic differences in the criteria for mRNA target prediction. For example, TargetScanS requires that the mRNA target site ends by an A residue located in complementarity to the first miRNA 5' nucleotide, whereas the two other algorithms allow any Watson-Crick base pairing in this position (97).

The reliability of these algorithms is sometimes questionable (see for example (106)). In order to reduce false-positive or false-negative targets of miRNAs, a useful tool, TargetCombo (<http://www.diana.pcbi.upenn.edu/cgi-bin/TargetCombo.cgi>), has been developed to combine many data sets. In particular it is possible to query files that combine all the targets predicted by at least one of the databases, Diana-microT, TargetScanS, miRanda and PicTar, or the targets all predicted by TargetScanS, miRanda and PicTar, or finally the results obtained with TarBase (105). The biocomputational tool TarBase (<http://diana.cslab.ece.ntua.gr/tarbase>) compiles all the experimentally validated targets (107, 108).

### ***Molecular mechanisms of translation regulation of mRNA targets by miRNAs***

#### ***mRNA maturation, stability, and translation: basic features***

After transcription, mRNAs are processed by addition of a cap m7G at the 5' end, a polyA extension at the 3' end, and by appropriate exon splicing. Cap and polyA tail strengthen mRNA stability since they interact with specific protein complexes that prevent exonuclease activity due to steric hindrance. mRNA stability is also regulated by the AU-rich elements binding proteins (ARE-BP), which bind to AU-rich domains located in the 3'UTR of mRNAs.

In the cytoplasm, mRNA translation requires three major steps: initiation, elongation, and termination (109, 110). The initiation occurs at the level of the cap, with first association of the 40S ribosomal subunit, methionine initiator tRNA, protein factors to aggregate in a 43S preinitiation complex, and involvement of the eIF4F complex. The 43S complex then shifts to the initiation codon AUG and partially dissociates, while a 80S is assembled by binding the 60S ribosomal subunit prior to translation.

Alternatively, translation initiation may rely on the recruitment of ribosomes by an internal ribosome entry site (IRES) and does not need the loading of the initiation protein complex at the mRNA cap structure (111). The IRES sequences are characterized by stem-loop secondary structures in the mRNA 5'UTR that contain an initiation codon at their 3' ends. The 40S ribosomal subunit binds directly to the initiation codon and initiates translation. After translation initiation, the ribosome moves codon by codon and recruits amino acid-loaded tRNAs. At the level of a stop codon, the action of termination factors promotes ribosome dissociation and translation stops. Translation initiation is a key step for translation regulation (110), and it is frequently repressed by miRNAs.

### **Translation initiation repression by miRNAs**

Several studies carried out on cultured cells demonstrated that the functional m7G cap is required for translation inhibition by miRNAs to take place. Indeed, miRNA inhibition of translation is impeded when mRNAs have a nonfunctional cap structure or when they are translated from IRES (112 , 113 ). Some mRNAs have a bicistronic feature, with one cistron translated in a cap-dependent way and another one translated from IRES. In this case, only the translation of the first cistron is repressed by miRNA (113 ). The importance of the m7G cap structure in the regulation of translation initiation is also illustrated by the fact that the central domain sequences of Ago proteins share some similarities with the central domain of the eIF4E factor, the cap-associated factor essential for translation initiation. In the Ago2 central domain, there are two phenylalanine residues that have proved to be necessary for binding Ago2 at the cap. Consequently, these proteins may inhibit initiation translation by competition with cap-associated factors such as eIF4E for binding to the cap (112 –114 ). In addition, translation initiation repression mediated by miRNA may rely on interaction between the miRISC complex and the eIF6 factor. This factor prevents the association between the 60S ribosomal subunit and the 40S ribosomal subunit and blocks translation initiation (115 ). The different mechanisms of the regulation of mRNA target expression by miRNAs are shown in Figure 12 .

### **Repression of the postinitiation step of translation by miRNAs**

Studies conducted on *C. elegans* highlighted that lin-14 and lin-28 mRNAs, the targets of the lin-4 miRNA, are associated with polysomes, while they are poorly translated into proteins (116 , 117 ). mRNA association with polysomes is usually a sign of strong translation activity. A low level of lin-14 and lin-28 mRNA translation could then be taken as an indication that miRNAs can play a role in translation inhibition at a postinitiation step. Similar situations were also observed in mammals. Indeed, Petersen *et al.* (118 ) reported that IRES-dependent translation of mRNA (a process that does not rely on a cap-dependent initiation step) can also be repressed by miRNAs. They found that miRNAs and their mRNA targets are present in translation-active polysomes and, in the presence of puromycin, which aborts the translation elongation step, miRNAs and mRNAs are finally found in the translation-inactive polysomes. It was then concluded that the postinitiation repression mechanism is due to a premature release of ribosomes (called drop off) during the elongation step (118 ). Postinitiation repression was also observed in HeLa cells in Kras mRNA, a let-7 target (119 ). Let-7 leads to inhibition of Kras expression and both the miRNA and its mRNA target were found associated with translation-active polysomes. However, the authors suggested that in this case, the miRNA-mediated postinitiation repression mechanism stems from a decrease in the speed of translational elongation. Finally, Nottrot *et al.* (120 ) also found miRNAs and mRNAs associated in polysomes, and concluded that translation repression by miRNA could be the result of a degradation of the newly synthesized polypeptide.

### **Regulation by degradation of the mRNA targets**

Several studies reported that miRNAs repress protein expression with limited effects on the levels of the target mRNAs. Nevertheless, other studies reported a miRNA-mediated decrease in stability of the mRNAs. For example, in *C. elegans* let-7 decreases lin-41 mRNA stability and lin-4 reduces the stability of both lin-14 and lin-28 mRNAs (121 ). In human, lin-28 mRNA stability is lowered by miR-125a and miR-125b (122 ). Moreover, in mammals miRNAs were reported to reduce protein output mainly by destabilization of target mRNAs (123 ). Accelerated deadenylation of the polyA-tailed target mRNA was reported to be a key step in miRNA-mediated mRNA decay (124 ).

In eucaryotes, deadenylation is first initiated by the PARN2/PARN3 complex and then requires a protein complex composed of NOT and two deadenylases, CAF1 and CCR4 (125 ). Once deadenylated, mRNAs can be degraded by exonucleases from their two ends. The 5'-to-3' end degradation is performed by the Xrn1 exonuclease after cap removal. In human, this step involves a complex made of DCP2 and several cofactors such as DCP1, EDC3, Ge-1, and RCK/p54 (126 ). All these proteins involved in the 5'-to-3' end degradation are located in cytoplasmic granules called p-bodies (processing bodies) (125 , 127 , 128 ), while the 3'-to-5' end mRNA degradation is carried out by protein complexes called exosomes (129 ).

These p-body cytoplasmic granules, also important in RNA interference phenomena, contain the GW182 protein, a 182-kDa protein containing glycine-tryptophane (GW) repeated elements (130 ). This protein is able to bind to Argonaute proteins, present in p-bodies associated with miRNAs and mRNA targets (131 ). Stress granules are another kind of cytoplasmic granule produced by eucaryote cells subjected to stress, such as oxidative stress, thermic shock, or UV irradiation (132 ). Using immunofluorescent techniques, stress granules and p-bodies were proved to be two independent and distinct structures (133 , 134 ). These cytoplasmic granules are a storage place for nontranslated mRNAs.

### **Translation activation by miRNAs**

In most of cases, miRNAs play a role in negative regulation of gene expression by mRNA target translation repression or by mRNA degradation. Nevertheless, in a few cases positive regulation by translation activation has been observed and especially in conditions of cellular stress. ARE sequences (AU-rich elements) located in the mRNA 3'UTR are critical elements for translation activation. It was shown that Ago2 and FXR1 bind to ARE sequences in order to activate the translation of the TNF $\alpha$  factor in cultured cells under serum privation and to stop the cell cycle (135 ). miR-369 which has a seed region complementary to two target sites of the ARE sequence of the

TNF $\alpha$  coding mRNA was shown to recruit Ago2 and FXR1 and to activate TNF $\alpha$  translation under serum privation conditions. It has been concluded that miRNAs may play a role of translation repressors in proliferating cells and a role of translation activators in cells blocked at the G0/G1 stage. Consequently, Ago2 may be involved in either activation or repression of mRNA translation (135).

Interestingly, mRNA translation activation by miRNA was also reported to proceed through an indirect action of miRNA by Eiring *et al.* (136). In the specific case of the chronic myelogenous leukemia, miR-328 was found acting as a decoy by binding to heterogeneous ribonucleoprotein hnRNP E2 in a miRNA's seed sequence independent way and this relieves the hnRNP E2 dependent translational repression of the CEBPA mRNA involved in myeloid cell differentiation.

miRNAs play an important role in translation regulation since it is assumed that up to 60% of the protein coding genes can be under the control of miRNAs (98). They are strongly involved in many cell processes such as development, differentiation, proliferation, and apoptosis (21, 137–140), all processes that are often deregulated in tumors. miRNA are believed to provide major contributions in tumorigenesis and description of these pathological roles at a molecular level are now increasingly documented.

## **miRNAs in tumorigenesis and tumor development processes**

### **Modifications of miRNA expression levels in cancer**

#### ***miRNA expression profiles in tumors***

Different techniques can be used to assess the miRNA expression level in tumor samples such as Northern blot analysis, quantitative PCR, oligonucleotide microarrays, SAGE and RAKE techniques, and quantitative flow cytometry or Next Generation Sequencing. Most of these techniques are useful for large-scale studies, involving large numbers of samples to be analyzed, or large numbers of different miRNAs to assay, or both.

Two of the early large-scale studies assayed many miRNAs in several types of tumors, using a quantitative flow cytometry technique based on the use of beads (141) or a microarray technique and Northern blots (142). Approximately 25% of the miRNAs studied were found to be significantly deregulated in at least one tumor type. The first study mainly revealed that miRNAs are underexpressed in tumor tissues in comparison to normal tissues, whereas the second one uncovered that miRNAs are overexpressed in tumor tissues. The different conclusions reported by these two studies conducted on similar types of tumor tissue (breast, colon, lung, pancreas, prostate, and stomach) probably resulted from the use of two different technical platforms or the sample size (142).

miRNA expression deregulation in several types of cancer were reassessed by taking into account the two above-mentioned original studies and the analysis of only one type of tumor (143). This highlighted that a number of miRNAs such as miR-21, miR-155, miR-221, and miR-222 appeared to be consistently overexpressed in many tumor tissues (brain, thyroid, gastrointestinal tract, liver, lung, and breast), whereas others such as miR-143, miR-145, and the let-7/miR-98 cluster were found to be underexpressed in tumor tissues. However, the expression of several miRNAs is not always modified identically in all types of cancer. For example, the mir-17/92 cluster has been reported to be overexpressed in seven tumor types and underexpressed in three others.

miRNAs are also interesting diagnostic biomarkers distinguishing different subtypes of tumors from the same tissue, such as in gliomas (144), thyroid cancer (145) or renal cell carcinoma (146).

#### ***Deregulation of miRNA expression by defects in the biogenesis mechanism***

Altered expression of the proteins involved in the miRNA biogenesis and maturation machinery has been observed in several cancers. For example, deregulation of Drosha expression is associated with ovarian cancer (147), skin epithelium cancer (148), and esophagus tumors (149). A high Drosha expression level is associated with a poor survival prognosis. Interestingly, when esophagus tumor cell lines were transfected by siRNAs designed to target the Drosha coding mRNA, a significant decrease in the cell proliferation was noted (149).

Inactivating mutations in XPO5, coding for Exportin 5, have been discovered in several cancer cell lines with microsatellite instability (150). These mutations lead to trapping pre-miRNAs in the nucleus, thus preventing miRNA processing.

Dicer was also found to be overexpressed in lung carcinoma and prostate adenocarcinoma (151, 152) and underexpressed in high-grade ovarian carcinoma, small-cell lung tumors with poor prognosis, and invasive lung adenocarcinoma (147, 152, 153). In addition, frameshift mutations in the TRBP2 genes were found to diminish the TRBP protein expression. This causes destabilization of the DICER complex and a defect in the processing of miRNAs (154).

Expression and activity of some major components of the miRNA processing pathway such as Drosha/DGCR8, Dicer/TRBP2 or Argonautes may be regulated by the transcription factors p53, p63, and p73 (155). The promoters of the genes coding for Drosha, DGCR8, Dicer and TRBP contain p53-RE sequences indicating that they could be direct transcriptional targets of p53 and preferentially of p63/p73. Moreover, direct interactions between WW domains in p63 and p73 and PY domains in DGCR8 protein may influence miRNA

processing. In addition, the pro-apoptotic TA-p63 isoform may indirectly decrease the expression of Dicer, for example, by increasing the expression of let-7, a miRNA that has been reported to reduce the expression of Dicer. Drosha, DGCR8, Dicer, and TRBP are also controlled by several miRNAs whose expression is regulated by p53. This suggests that p53/p63/p73 could regulate the expression of the miRNA processing components, at both the transcriptional and post-transcriptional levels.

#### ***Deregulation of miRNA expression by chromosomal rearrangements***

Two studies highlighted that miRNA genes are located in chromosomal regions that are frequently altered in cancers (156, 157). Alterations of the copy number for 37%, 73%, and 86% of 283 miRNA coding genes were pinpointed in ovarian cancer, breast cancer, and melanoma, respectively. Nearly 40 miRNA coding genes are altered in common in these three types of tumor (157). Modifications of the expression of miRNA coding genes may result from chromosomal translocation, leading, for example, to overexpression of the gene coding for miR-125b, due to translocation t(2;11) in some types of leukemia (158). They may also be linked to amplification or deletion of genomic regions. This is exemplified in the 17q23 region, which was found to be amplified in neuroblastoma (159). This region hosts the gene coding for miR-21, which is usually upregulated in tumors. Another example is the presence of a deletion in the 13q14 region in several cases of B-cell chronic lymphoid leukemia. Genes coding for miR-15a and miR-16-1, underexpressed in 68% of these types of tumor, are located in this region (160).

#### ***Deregulation of miRNA expression due to point mutations in coding genes***

The number of point mutations or SNPs (single nucleotide polymorphisms) in human pri-miRNA or pre-miRNA sequences has been reported to be usually very low (161). Nevertheless, several studies have indicated that SNPs may explain changes in miRNA expression between tumor and normal tissues. One SNP (G:C) in the pre-miR-146a sequence (60 nucleotides from the first nucleotide) has been revealed and associated with a decrease in the expression of mature miR-146a in thyroid papillary carcinoma (162) and in prostate cancer (163). Moreover, a G/C heterozygous status in tissues is associated with a higher risk of thyroid papillary carcinoma in comparison to G/G or C/C homozygous status (162). In prostate cancer, a C/C homozygous status was reported to correlate to halving the risk of cancer in comparison to G/G or G/C genotypes (163). Another SNP (G:U), identified in the seed region of miR-125a located 8 nt from the 5' end, could have an impact on the interaction between miRNA-125a and its mRNA target (164). A G/U heterozygous status was observed in some cases of breast cancer (165). In non-small-cell lung cancer, an SNP (U:C) in the pre-miR-196a-2 (78 nucleotides from the first nucleotide) has been identified and patients with a C/C homozygous status had a shorter survival rate compared to patients with a U/U or U/C genotype (166). Calin *et al.* highlighted mutations in five out of 42 miRNAs in 15% of chronic lymphocytic leukemia (CLL) samples (167). In another type of lymphocytic leukemia, MLL-AF4 acute lymphocytic leukemia, miR-128b has been found to be underexpressed in tumor samples, due to a mutation in the primary transcript located 13 bp from the 3' end of mature miR-128b (168). This mutation also contributes to glucocorticoid resistance of tumor cells.

#### ***Modifications of miRNA expression due to transcriptional regulation***

Transcription of miRNA encoding genes by RNA polymerase II can be regulated by several transcriptional factors. In the flanking regions of miRNA-coding genes in *C. elegans* and in humans the presence of patterns involved in transcriptional regulation has been studied (169). A number of transcriptional factors such as p53, E2F, and Myc have been reported to play a role in this regulation.

p53, known to be a tumor suppressor, has been highlighted as a transcriptional regulator of several miRNAs (170). It acts as a transcription activator of the miR-34a gene, as it can bind to the promoter region of this gene (171–173). It may also impair the expression of several miRNAs by inhibition of the transcription factor E2F1 (174). Activation by transcription factors belonging to the E2F family has been reported to increase the expression of some miRNAs (175). Another transcription factor, Myc, which has oncogenic properties, has been described as an expression modulator for a number of miRNAs. Indeed, by binding to promoter regions, Myc has been shown to be an expression activator of the miR-17/92 cluster, for example (176), but also an expression repressor for many miRNAs involved in proliferation processes (177).

Transcription regulation, which can account for a modulation of miRNA expression levels, can also result from epigenetic modifications at the promoter regions of specific miRNA coding genes. One important epigenetic modification is DNA methylation, which is a reversible phenomenon and involves only cytosines belonging to CpG dinucleotide motifs. CpG-rich genomic domains, called CpG islands, can be located in several promoter regions. DNA methylation of CpG islands in promoters is a phenomenon that controls the expression level of downstream genes, as this modifies the chromatin structure and consequently the binding of transcription factors; in addition it favors MBD (methyl-DNA binding) protein fixation (178).

The role of DNA methylation in repression of miRNA expression was confirmed by using DNA demethylation drugs on cultured tumor cells that promote miRNA overexpression (179–181). Several miRNAs have been studied more specifically, such as miR-124 (181) and miR-34a (182), for which the lower expression in cell lines or in tumor tissues is due to a hypermethylation of CpG islands, and miR-128 (183) and let-7a-3 (184), which are overexpressed due to a hypomethylation of CpG islands.



## Modifications of the interactions between miRNAs and their mRNA targets

### *Deregulation by chromosomal modification*

As mentioned above, several genomic alterations, such as chromosomal rearrangements or point mutations, may rationalize the differences observed in miRNA expression between tumor and normal tissues. These alterations are important events in the tumor genesis process, as they prevent proper regulation of normal cellular processes by miRNAs. In addition, and similarly, genomic alterations can also affect the 3'UTR sequences of mRNA targets and lead to gene regulation modifications by impairment of the miRNA/mRNA target hybridizations.

One study demonstrated that a 4-nt TTCA insertion in the 3'UTR of the IL-1 alpha mRNA significantly disrupts a binding site for miR-122 and miR-378 and has a substantial influence on IL-1 alpha expression regulation (185). Studies conducted on mantle cell lymphoma have shown high expression of CCND1 in these tumors due to deletions and mutations in the CCND1 mRNA 3'UTR (186). These sequence modifications impair the interaction between CCND1 mRNA and miR-16, increasing the expression of the CCND1 protein (187). Genomic translocation can delete the normal 3'UTR part of the mRNA coding for HMGA2, making let-7 unable to bind to this region and to restrain HMGA2 expression (188). This protein has been shown to be involved in the tumorigenesis of many malignant tumors such as lung cancer (189–191) and pancreas cancer (192).

In the case of ABCG2 (a ubiquitous ATP-binding cassette (ABC) transporter, playing a role in absorption, distribution, and elimination of drugs), a shortening of the 3'UTR mRNA has been detected in drug-resistant colon cancer cell lines, leading to a loss of a miR-519c binding site. This relieves the repression of ABCG2 translation, contributes to the overexpression of the transporter, and confers drug resistance to the tumor cells (193).

### *Deregulation by point mutations*

Yu *et al.* (194) investigated SNPs in the mRNA 3'UTR sequence complementary to several miRNAs. They noted fewer SNPs in the regions that are complementary to the miRNA seed region than in the other part of the 3'UTRs (194). This was taken as evidence of a selection pressure and the importance of the regulatory mechanism by miRNAs.

SNPs located in miRNA complementary regions have been studied in a tumor context in an increasing number of reports. Two SNPs in the 3'UTR of the KIT mRNA (tyrosine kinase receptor) in thyroid papillary carcinoma have been described. These SNPs (G:A and G:C) affect the binding of miR-221/222 and miR-146a/146b miRNAs to the KIT mRNA, favor expression of the protein, and consequently increase the risk of cancer (195). In CD86 mRNA 3'UTR, a (G:C) SNP was found associated with a higher risk of sporadic colorectal cancer. This site is the target of five miRNAs; three experience a decrease in their binding affinity with the mRNA, contrary to the other two (196). Another study was conducted on the mRNA 3'UTR region coding for KRAS in non-small-cell lung cancer in moderate smokers. This mRNA contains ten target sites in the 3'UTR for the miRNA let-7 family. The authors described only one SNP per target site and most of these SNPs are infrequently found in tumors (197). Only one SNP (U:G) was found in about 20% of tumors and therefore can be associated with a higher risk of developing these tumors, and this type of mutation leads to an increase in KRAS mRNA expression *in vitro* (198). Another study was conducted on breast cancer and most particularly on integrins, proteins involved in the control of cellular attachment to the extracellular matrix. SNPs were found in the 3'UTR of integrin coding mRNAs, in particular ITGB4. A strong correlation was found between the presence of the polymorphic allele and the negative status of hormonal receptors in breast cancer (199).

## Oncogene and tumor suppressor miRNAs involved in molecular pathways of tumor development

miRNAs can be considered as oncogenes when an increase in their expression contributes to a malignant transformation of normal cells, or on the contrary, as tumor suppressors in the reciprocal. The status of oncogenes or tumor suppressors is generally given to miRNAs that have been found to be deregulated in many tumor types. Several miRNAs have been described as oncogenes (frequently called oncomiRs). A few specific examples are given hereafter.

miR-372/373 are two miRNAs that have been described as playing a role in the development of testicular germ cell tumors in presence of active wild-type p53 and oncogenic RAS (200). The polycistronic cluster miR17-92 also seems to have an oncogenic role such as in lung cancer and B-cell lymphoma (201, 202). Indeed, overexpression of this cluster seems to accelerate tumor development of B-cell lymphoma in mice expressing c-myc. miR-155 is also described as an oncogenic miRNA, as it is overexpressed in B-cell lymphoma (142, 203–206). Some miRNAs, such as miR-21, are also considered as oncogenes when they can repress the expression of tumor suppressor proteins. miR-21 is overexpressed in many tumor types and targets the mRNAs of tumor suppressors PDCD4 and TPM1 (143, 207–210). On the contrary, other miRNAs have been described as tumor suppressors: let-7 (156, 189, 211–216) as well as miR-15a and miR-16-1 (160, 217–220). miR-34a is also considered a tumor suppressor repressing several oncogenic genes, such as c-Met in brain tumors (221) and uveal melanoma (222) or Bcl-2 in neuroblastoma (223).

For a more comprehensive list of oncogenic or tumor suppressor miRNAs, the reader is referred to the miR 2 Disease database ([www.mir2disease.org](http://www.mir2disease.org) (224)).

miRNAs involved in tumor development can control many key signaling pathways such as apoptosis, cellular proliferation, and angiogenesis.

### ***Apoptosis and cellular proliferation processes***

The apoptosis process can be activated *via* two pathways, the intrinsic and the extrinsic pathways (Figure 13 ). In the intrinsic one, several pro-apoptotic and anti-apoptotic members of the Bcl-2 family are involved and their actions are antagonized to regulate mitochondrial membrane permeability. The anti-apoptotic proteins Bcl-2 and Bcl-xL are often overexpressed in tumors (225 –227 ) and prevent mitochondrial permeabilization by inhibition of the cytosolic pro-apoptotic Bax and Bak (228 ). After a cellular stress, expression of pro-apoptotic members of the Bcl-2 family such as Puma and Noxa is enhanced by p53-dependent transcriptional activation. Subsequent activation of Bax and Bak proteins then allow their migration to the mitochondrial membrane where they concur to increase the permeability of the mitochondrial membrane. This permeabilization is associated with a release of cytochrome *c* into the cytoplasm ( 229 ), which binds to the APAF-1 protein (apoptotic protease-activating factor-1) and to two procaspases-9 to form the apoptosome. Then this protein complex promotes the activation of caspase-9, an apoptosis-initiating caspase (230 ). Activated caspase-9 cleaves procaspase-3, -6, or -7. In normal conditions, activation of these caspases is inhibited by proteins called IAPs (inhibitor of apoptosis protein). IAPs are inhibited by Smac/DIABLO proteins that are released into the cytoplasm after membrane permeabilization of mitochondria (231 , 232 ).

As for the extrinsic apoptotic pathway, it starts outside of the cell with an activation of the pro-apoptotic receptors at the surface of the cell by ligands such as CD95L (FAS-L). Ligand binding to the extracellular domain receptors promotes intracellular domain binding to the FADD protein (Fas-associated death domain) (233 ). Then this complex recruits procaspase-8 or -10 and activates it. Activated caspases-8 and -10 then participate in the downstream steps of the intrinsic apoptotic pathway as they activate activator caspase-3, -6, or -7 and can also cleave and activate the Bid protein. The truncated protein tBid allows the relocalization of Bax and Bak (234 ).

A number of miRNAs have been shown to be able to regulate the apoptotic pathways at different stages (235 ). Several proteins of the Bcl-2 family are regulated by miRNAs. The Bcl-2 mRNA is the target of miR-15a/16 and it was demonstrated that both miRNAs negatively regulate Bcl-2 at a post-transcriptional level and induce apoptosis (236 ). The Bcl-xL protein is inhibited by the pro-apoptotic protein Bim whose expression level is downregulated by certain miRNAs of the miR-17/92 and miR-106b/25 clusters. Indeed, the miR-17/92 cluster is essential for B cell development, and absence of miR-17/92 leads to increased levels of the pro-apoptotic protein Bim and inhibits B cell development (237 ). miR-17/92 was found to be overexpressed in many cancers such as B-cell lymphoma. The miR-106b/25 cluster was found to be overexpressed in many gastric and prostate cancers. Some miRNAs, most particularly miR-106b, are assumed to target Bim but also p21, which is involved in cell cycle control, and E2F1 (238 , 239 ). miR-34a is also an important regulator of the initiation of the apoptosis pathway, as this miRNA lowers the expression of the deacetylase SIRT1. This leads to the acetylation of p53, the increase in its transcriptional activity, and the induction of the pro-apoptotic protein Puma (240 ). A recent study showed that Puma is the target of two miRNAs, miR-221/222, in glioblastoma cells (241 ). These two miRNAs also play an important role in cellular proliferation by regulation of p27<sup>Kip1</sup> expression (242 ).

The second part of the intrinsic pathway, with apoptosome formation and activation of caspases, is also regulated by miRNAs. For example, miR-1 and miR-133 are co-transcribed from the same polycistron, but they have antagonistic roles in apoptosis, as miR-1 has a pro-apoptotic effect, whereas miR-133 is an anti-apoptotic factor. In fact, miR-1 targets the anti-apoptotic proteins HSP60 and HSP70, two proteins that prevent apoptosome formation, while miR-133 represses caspase-9 expression (243 ). miR-21 also plays an anti-apoptotic role initially observed in glioblastoma cells (244 ), since this miRNA regulates the expression of APAF-1, caspase-3, and PDCD4 (245 ). In addition, PDCD4 also regulates caspase-3 activity (246 ).

The extrinsic pathway can be the target of miR-21 and miR-182, which control the expression of FAS-L (247 ) and protein FADD ( 248 ), respectively.

### ***Angiogenesis process***

Growth of new blood vessels from preexisting vessels, a process called angiogenesis, is a key step in tumor progression (249 , 250 ). The control of this process by miRNAs has been evidenced to occur after the experimental knockdown of Dicer, by the transfection of specific siRNAs in endothelial cells. Indeed, the reduction of the expression of Dicer led to a decrease in angiogenesis, by a reduction of capillary sprouting of endothelial cells and tube-forming activity (251 ). After Dicer knockdown, Suarez *et al.* have shown an alteration in the expression of key genes in angiogenesis such as IL-8, angiopoietin receptor Tie-2, and VEGFR2 (252 ). Several studies analyzed the expression levels of miRNAs in endothelial cells and highlighted their pro-angiogenic properties *in vivo* (miR-126, miR-378, miR-296, and the miR-17/92 cluster), or *in vitro* (miR-210, let-7f, miR-27b, and miR-130a), or their anti-angiogenic properties (miR-221/222, miR-15b, and miR-16) (253 ). miR-126, the only endothelial cell-specific miRNA, was studied *in vivo* in a mouse model in which a drop in the expression of this miRNA was correlated with a decrease in angiogenesis and vascular integrity (254 ). The same conclusion was

drawn in zebrafish in which knockdown of miR-126 leads to the loss of vascular integrity. This miRNA plays a role in the repression of negative regulators of the VEGF pathway, such as SPRED1 and PIK3R2, and it also seems that miR-126 regulates the expression of EGFL7 (255). This protein is also involved in many processes and particularly in angiogenic sprouting (256).

Other miRNAs, nonspecific of endothelial cells, such as miR-378, miR-296, and the miR-17/92 cluster, also have pro-angiogenic properties. The overexpression of miR-378 in glioblastoma U87 cells leads to angiogenesis of tumors after injection of the cells in nude mice by repression of the expression of tumor suppressors Fus-1 and SuFu (257). An inhibition of miR-296 in the glioblastoma U87 cells injected in nude mice reduces angiogenesis (258). Concerning the miR-17/92 cluster, overexpression in cells promotes the development of better-perfused tumors (259).

miR-210, a hypoxia-inducible miRNA, stimulates the cellular migration and the formation of vessel-like structures by downregulating Ephrin-A3 expression (260). miR-130a shows pro-angiogenic properties by targeting mRNAs coding for the anti-angiogenic proteins Gax and Hoxa5 (261). On the contrary, some miRNAs are more likely anti-angiogenic factors such as miR-221/222, whose overexpression *in vitro* in endothelial cells leads to a decrease in cellular migration and the formation of vessel-like structures by repression of c-Kit (262). The miRNAs miR-15b and miR-16 target the VEGF-coding mRNA and thus have anti-angiogenic properties (263).

### **Exosomal miRNAs: new potential tumor biomarkers**

Quantitative analyses of miRNA expression in tumor tissues revealed that miRNAs can be regarded as appropriate biomarkers that can help discriminate tumor types. miRNA profiling for tumor typing appears to be an attractive approach for diagnosis as a complement to pathology methods (144). This can be performed directly on tumor tissue specimens, but other less invasive approaches are currently under investigation. These approaches are based on the fact that miRNAs can be found in detectable amounts in small membrane vesicles (about 100 nm in diameter) called exosomes (264). Exosomes offer miRNAs a protection against RNases (265) and are secreted by a wide range of cell types such as hematopoietic cells, dendritic cells, mast cells, neurons, and tumor cells (266–268). Exosome purification and analysis of miRNA content may give new opportunities for diagnosis. In addition, exosomes and their RNase-protected miRNA are present in several biological fluids. Indeed, their presence has been evidenced in blood (268–272), urine (273, 274), saliva (275, 276) and amniotic fluid (277).

Assaying exosomal miRNAs in body fluids could be useful for cancer diagnosis. In fact, tumor-derived exosomes were proved to contain numerous tumor-specific miRNAs. For instance, exosomes can be enriched either in the let-7 miRNA family if derived from metastatic gastric cancer (278) or in various pre-miRNAs if derived from mesenchymal stem cells (279). miRNAs derived from glioma exosomes can therefore be envisioned for use as diagnosis biomarkers or surrogate markers for therapy follow-up (268, 269). For example, the exosomal miRNAs miR-21 and miR-155 extracted from blood have been reported to be valuable biomarkers for diagnosis of patients with lung adenocarcinomas (271). In ovarian cancer, exosomal circulating miRNAs can also be informative to distinguish various tumor stages (280). In prostate cancer cells, irradiation-induced premature senescence is associated with an increased release of exosomes. Thus, a quantitative assessment of the tumor exosomes released in body fluids should constitute an easy means for therapy follow-up (281).

It should be noted that exosomes may also have considerable implications in cancer development, given that these vesicles have been reported to be involved in an intercellular transfer of the mRNAs and miRNAs they contain between glioma cells (282), or from tumor cells into normal cells in the tissues (264, 283). Also, exosomes derived from cancer stem cells, which seem to be responsible for the increase in tumor growth, invasion, or vascularization, could establish a premetastatic niche away from the tumor tissue (284).

### **miRNAs in cancer therapy**

With the discovery of the role of miRNAs in controlling the expression of many proteins, and moreover that they behave as oncogenic or tumor suppressor components, it soon became clear that miRNAs can be involved or may be used in new therapeutic approaches. It goes without saying that taking advantage of miRNAs in new antitumor developments requires that a number of hurdles be overcome (285).

Briefly, miRNAs intrinsically have a number of attractive features for therapy. Their mechanism of action guides the therapeutic process. In comparison to small inhibitory molecules that reduce the activity of targeted enzymes but leave the amount of cellular enzyme intact, miRNAs can be used to reduce the cellular concentration of targeted proteins. miRNAs are natural cellular components and they include an intrinsic signature that may ensure specificity to target. These may have important consequences in the control of side effects. Nevertheless, accurate miRNA-target recognition is not perfectly managed (see above) and off-target effects are still dreaded. On the other hand, one single miRNA can target several oncogenic proteins and the opportunity to simultaneously repress several oncogenes with only a single miRNA appears very attractive in the search to improve anticancer therapy, while limiting possible side effects. An example of this multitarget therapy can be found with miR-34a, which represses c-Met, CDK4 (cyclin-dependent kinase 4), and Bcl-2 (B-cell lymphoma 2), all involved in separate oncogenic pathways (286).

miRNA-based therapies can be tentatively classified according to two different criteria: the antitumoral effects that are strategically expected and the mode of action triggered. First and most specifically, several antitumoral effects can be putatively promoted using miRNA-based strategies (287). Indeed, given the numerous roles played by miRNAs in the tumor physiopathology mentioned above, modulation of miRNA expression in tumor tissues may have direct effects on several biochemical pathways and proliferation, survival, differentiation, and migration of tumor cells. Hence therapeutic strategies can include direct tumor suppressive effects, antiangiogenic effects, antimetastatic effects, suppression of immune evasion of tumors, or sensitization of tumor cells to traditional anticancer treatments such as radiotherapy or chemotherapy.

Second, miRNA-based therapies can be based on the development of miRNA mimicking compounds to increase the cellular concentration of any given miRNA or on the contrary of miRNA antagonists to reduce their level. Upregulation of miRNA cellular concentration can be obtained by transfection of the cells with exogenous miRNA, *i.e.*, pre-miRNA or mature miRNA, whereas antisense-mediated inhibition is based on the use of complementary antisense oligonucleotides of mature miRNAs to downregulate miRNAs (287). A third attractive therapeutic approach called indirect miRNA-based therapy takes advantage of the fact that some miRNAs are expressed in reduced amounts in cancer cells but are present in high concentration in normal cells. The introduction of specific vectors or constructs containing a miRNA-regulated suicide gene in cancer cells may result in the expression of the given suicide gene only in the cancer cells that are devoid of the suicide gene-regulating miRNA. This strategy may be very safe and leave all normal cells unaffected.

miRNA replacement is the approach based on the administration of synthetic miRNAs so as to increase the cellular concentration of tumor suppressor miRNAs whose levels are frequently lowered in tumors. For example, in the case of let-7, which is a tumor suppressor downregulated in several cancer types, an intratumoral introduction of the let-7 oligonucleotide into a mouse model of non-small-cell lung cancer led to a reduction of the tumor size (288). Exogenous delivery of a miR-34 mimic by transfection or use of lentivirus constructs resulted in a reduction of the tumor in gastric cancer (289). miR-based strategies are under investigation at a preclinical stage for development of miRNA replacement therapies against cancers. For example, several are based on the delivery of mimics of miR-34, miR-16 or let-7, which are said to be tumor suppressors in tumors (Mirna Therapeutics, Austin, TX, USA) (286).

Antagonists of miRNAs are required to reduce the cellular levels of oncogenic miRNAs that are overexpressed in tumors. This can be achieved using antisense oligonucleotides (ASO) that can hybridize to miRNAs and then prevent miRNA interactions with the mRNA targets. Chemical modifications are needed to protect ASOs from nuclease degradation and to improve their affinity for miRNAs (290). The main modifications are the use of 2'-O-methyl-RNA nucleotides or locked nucleic acid (LNA™) nucleotides. In the locked nucleic acid nucleotides, the ribose ring is modified by a methylene bridge connecting the 2'-O atom and the 4'-C atom. A recent study has successfully used 2'-O-methyl- and DNA/LNA-mixed oligonucleotides to specifically knock down miR-21 and to investigate the potential contribution of this miRNA in the regulation of apoptosis-associated genes in glioblastoma cell lines (244). In these cell lines, Papagiannakopoulos *et al.* compared these two miR-21 downregulating types of ASOs and observed greater knockdown using LNA-complementary antisense miR-21 than the 2'-O-methyl-complementary antisense miR-21, increasing the levels of major components of the apoptotic pathway (245).

miR-122 is a miRNA expressed in liver that is essential for replication of the hepatitis C virus (HCV) RNA genome, causing liver disease and increased risk of hepatocellular carcinoma. Therapeutic silencing of miR-122 using a locked nucleic acid (LNA)-modified oligonucleotide (SPC3649) complementary antisense to miR-122 was proved to suppress viremia in primates (291). The antgomiR-122 (SPC3649 or Miravirsin - Santaris Pharma A/S, Hørsholm, Denmark) is currently being evaluated in Phase 2a clinical trials. Regulus Therapeutics (San Diego, CA, USA) is developing proprietary chemically modified anti-miR oligonucleotides delivered systemically to target miR-21, which is overexpressed in many cancer types (142) and can promote tumor progression and metastasis (292). Regulus's anti-miR-21 downregulates miR-21 in the liver and reduces hepatocellular carcinoma (HCC).

Sensitization of tumors to chemotherapy or radiotherapy by miRNA-based therapy also seems to be a valuable approach according to a number of published results. For example, the oncogenic cluster miR-17/92 has been shown to be involved in the radioresistance of tumor cells. Indeed, overexpression of these clustered miRNAs in mantle cell lymphoma cells decreases the radiosensitivity of these cells by targeting PTEN and activating the PI3K/Akt signal pathway (293). In a similar way, miR-221/222 are also described as being involved in the radioresistance of tumor cells in gastric cancer. A knockdown of these miRNAs in cancer cell lines using complementary antisense oligonucleotides (2'-O-methyl-oligonucleotides) increases PTEN expression and increases the radiosensitivity of the tumoral cells (294).

The role played by miRNAs in the chemoresistance process ((295) for review) and as biomarkers of chemoresistance has been demonstrated. Ranade *et al.* found that miR-92\*, miR-147, and miR-574-5p are overexpressed in small-cell lung cancer and are potential biomarkers of chemoresistance (296). In other types of cancer such as colon cancer or ovarian cancer, several other miRNAs have been described as being involved in chemoresistance. It has been shown that miR-140 and miR-215 are overexpressed in drug-resistant colorectal cancer cell lines and the targeted proteins involved in this resistance may be HDAC4 and DHFR, respectively (297, 298). In ovarian cancer, several miRNAs are involved in drug resistance, such as miR-30c, miR-130a, and miR-335, which are downregulated in

drug-resistant cell lines and promote upregulation of M-CSF (macrophage colony-stimulating factor), a previously described resistance factor for ovarian cancers (299).

Collectively, all these studies show that appropriate control of specific miRNA concentrations in tumors may have beneficial impacts on tumor initiation and promotion. One main obstacle to these therapeutic strategies, as pointed by Purow (285), probably relates to the correct delivery of the miRNA mimics or antagonists to tumor cells.

## Conclusion

miRNA appears to be an original class of noncoding RNAs. Approximately 1000 different miRNAs are expressed in human. This rather limited number may facilitate the understanding of their global physiological or pathological roles in cells. However, the large number of mRNAs that they can individually target makes the analysis extremely complicated. Moreover, additional factors or events probably remain to be identified to achieve a complete appraisal of the way mRNA expression is finely tuned in cells under the control of miRNAs.

Study of miRNAs in cancer obviously provides new opportunities for diagnosis. On the other hand, given their critical role in the control of gene expression and in their possible involvement in molecular dialogs between cells driven by exosomes, research programs in which miRNAs are regarded as targets for new therapeutic drugs or approaches seem perfectly warranted and look promising.

## Acknowledgements:

This study was supported by grants from the Ligue contre le Cancer (comités départementaux Isère, Drôme and Ardèche and Ligue Nationale) (<http://www.ligue-cancer.net/>); the Région Rhône-Alpes (<http://www.rhonealpes.fr/>); the Lyon, Auvergne, Rhône-Alpes Cancéropole (<http://www.canceropole-clara.com/>). We thank Ms. L. Northrup (PhD, ELS; English Solutions) for manuscript editing.

## Abbreviations

miRNA : microRNA

siRNAs : short interfering RNA

MiRISC : miRNA-induced silencing complex

## References:

1. Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell*. 75 : 843 - 854 1993 ;
2. Heywood SM, Kennedy DS, Bester AJ. Separation of specific initiation factors involved in the translation of myosin and myoglobin messenger RNAs and the isolation of a new RNA involved in translation. *Proc Natl Acad Sci U S A*. 71 : 2428 - 31 1974 ;
3. Wightman B, Ha I, Ruvkun G. Posttranscriptional regulation of the heterochronic gene *lin-14* by *lin-4* mediates temporal pattern formation in *C. elegans*. *Cell*. 75 : 855 - 862 1993 ;
4. Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G. The 21-nucleotide *let-7* RNA regulates developmental timing in *Caenorhabditis elegans*. *Nature*. 403 : 901 - 906 2000 ;
5. Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kuroda MI, Maller B, Hayward DC, Ball EE, Degnan B, Müller P, Spring J, Srinivasan A, Fishman M, Finnerty J, Corbo J, Levine M, Leahy P, Davidson E, Ruvkun G. Conservation of the sequence and temporal expression of *let-7* heterochronic regulatory RNA. *Nature*. 408 : 86 - 89 2000 ;
6. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. *Science*. 294 : 853 - 858 2001 ;
7. Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W, Tuschl T. Identification of tissue-specific microRNAs from mouse. *Curr Biol*. 12 : 735 - 739 2002 ;
8. Reinhart BJ, Weinstein EG, Rhoades MW, Bartel B, Bartel DP. MicroRNAs in plants. *Genes Dev*. 16 : 1616 - 1626 2002 ;
9. Aravin AA, Lagos-Quintana M, Yalcin A, Zavolan M, Marks D, Snyder B, Gaasterland T, Meyer J, Tuschl T. The small RNA profile during *Drosophila melanogaster* development. *Dev Cell*. 5 : 337 - 350 2003 ;
10. Dostie J, Mourelatos Z, Yang M, Sharma A, Dreyfuss G. Numerous microRNPs in neuronal cells containing novel microRNAs. *RNA*. 9 : 180 - 186 2003 ;
11. Houbaviy HB, Murray MF, Sharp PA. Embryonic stem cell-specific MicroRNAs. *Dev Cell*. 5 : 351 - 358 2003 ;
12. Lagos-Quintana M, Rauhut R, Meyer J, Borkhardt A, Tuschl T. New microRNAs from mouse and human. *RNA*. 9 : 175 - 179 2003 ;
13. Lim LP, Glasner ME, Yekta S, Burge CB, Bartel DP. Vertebrate microRNA genes. *Science*. 299 : 1540 - 1540 2003 ;
14. Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ. miRBase: tools for microRNA genomics. *Nucleic Acids Res*. 36 : D154-158 - D154-158 2008 ;
15. Griffiths-Jones S. The microRNA Registry. *Nucleic Acids Res*. 32 : D109-111 - D109-111 2004 ;
16. Place RF, Li LC, Pookot D, Noonan EJ, Dahiya R. MicroRNA-373 induces expression of genes with complementary promoter sequences. *Proc Natl Acad Sci U S A*. 105 : 1608 - 13 2008 ;
17. Gonzalez S, Pisano DG, Serrano M. Mechanistic principles of chromatin remodeling guided by siRNAs and miRNAs. *Cell Cycle*. 7 : 2601 - 8 2008 ;
18. Kim DH, Saetrom P, Snove O Jr, Rossi JJ. MicroRNA-directed transcriptional gene silencing in mammalian cells. *Proc Natl Acad Sci U S A*. 105 : 16230 - 5 2008 ;
19. Khraiweh B, Arif MA, Seumel GI, Ossowski S, Weigel D, Reski R, Frank W. Transcriptional control of gene expression by microRNAs. *Cell*. 140 : 111 - 22 2010 ;
20. Watanabe T, Totoki Y, Toyoda A, Kaneda M, Kuramochi-Miyagawa S, Obata Y, Chiba H, Kohara Y, Kono T, Nakano T, Surani MA, Sakaki Y, Sasaki H. Endogenous siRNAs from naturally formed dsRNAs regulate transcripts in mouse oocytes. *Nature*. 453 : 539 - 543 2008 ;
21. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 116 : 281 - 297 2004 ;
22. Malone CD, Brennecke J, Dus M, Stark A, McCombie WR, Sachidanandam R, Hannon GJ. Specialized piRNA pathways act in germline and somatic tissues of the *Drosophila* ovary. *Cell*. 137 : 522 - 535 2009 ;
23. Li C V, Vagin V, Lee S, Xu J, Ma S, Xi H, Seitz H, Horwich MD, Syrzycka M, Honda BM, Kittler EL, Zapp ML, Klattenhoff C, Schulz N, Theurkauf WE, Weng Z, Zamore PD. Collapse of germline piRNAs in the absence of Argonaute3 reveals somatic piRNAs in flies. *Cell*. 137 : 509 - 21 2009 ;
24. Yan Z, Hu HY, Jiang X, Maierhofer V, Neb E, He L, Hu Y, Hu H, Li N, Chen W, Khaitovich P. Widespread expression of piRNA-like molecules in somatic tissues. *Nucleic Acids Res*. 39 : 6596 - 607 2011 ;
25. Aravin A, Gaidatzis D, Pfeffer S, Lagos-Quintana M, Landgraf P, Iovino N, Morris P, Brownstein MJ, Kuramochi-Miyagawa S, Nakano T, Chien M, Russo JJ, Ju J, Sheridan R, Sander C, Zavolan M, Tuschl T. A novel class of small RNAs bind to MILI protein in mouse testes. *Nature*. 442 : 203 - 207 2006 ;

- 26 . Girard A , Sachidanandam R , Hannon GJ , Carmell MA . A germline-specific class of small RNAs binds mammalian Piwi proteins . *Nature* . 442 : 199 - 202 2006 ;
- 27 . Grivna ST , Beyret E , Wang Z , Lin H . A novel class of small RNAs in mouse spermatogenic cells . *Genes Dev* . 20 : 1709 - 1714 2006 ;
- 28 . Watanabe T , Takeda A , Tsukiyama T , Mise K , Okuno T , Sasaki H , Minami N , Imai H . Identification and characterization of two novel classes of small RNAs in the mouse germline: retrotransposon-derived siRNAs in oocytes and germline small RNAs in testes . *Genes Dev* . 20 : 1732 - 1743 2006 ;
- 29 . Rodriguez A , Griffiths-Jones S , Ashurst JL , Bradley A . Identification of mammalian microRNA host genes and transcription units . *Genome Res* . 14 : 1902 - 1910 2004 ;
- 30 . Saini HK , Enright AJ , Griffiths-Jones S . Annotation of mammalian primary microRNAs . *BMC Genomics* . 9 : 564 - 564 2008 ;
- 31 . Kim VN , Han J , Siomi MC . Biogenesis of small RNAs in animals . *Nat Rev Mol Cell Biol* . 10 : 126 - 139 2009 ;
- 32 . Cai X , Hagedorn CH , Cullen BR . Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs . *RNA* . 10 : 1957 - 1966 2004 ;
- 33 . Lee Y , Kim M , Han J , Yeom KH , Lee S , Baek SH , Kim VN . MicroRNA genes are transcribed by RNA polymerase II . *EMBO J* . 23 : 4051 - 4060 2004 ;
- 34 . Megraw M , Baev V , Rusinov V , Jensen ST , Kalantidis K , Hatzigeorgiou AG . MicroRNA promoter element discovery in Arabidopsis . *RNA* . 12 : 1612 - 1619 2006 ;
- 35 . Saini HK , Griffiths-Jones S , Enright AJ . Genomic analysis of human microRNA transcripts . *Proc Natl Acad Sci U S A* . 104 : 17719 - 17724 2007 ;
- 36 . Zhou X , Ruan J , Wang G , Zhang W . Characterization and identification of microRNA core promoters in four model species . *PLoS Comput Biol* . 3 : e37 - e37 2007 ;
- 37 . Marson A , Levine SS , Cole MF , Frampton GM , Brambrink T , Johnstone S , Guenther MG , Johnston WK , Wernig M , Newman J , Calabrese JM , Dennis LM , Volkert TL , Gupta S , Love J , Hannett N , Sharp PA , Bartel DP , Jaenisch R , Young RA . Connecting microRNA genes to the core transcriptional regulatory circuitry of embryonic stem cells . *Cell* . 134 : 521 - 533 2008 ;
- 38 . Ozsolak F , Poling LL , Wang Z , Liu H , Liu XS , Roeder RG , Zhang X , Song JS , Fisher DE . Chromatin structure analyses identify miRNA promoters . *Genes Dev* . 22 : 3172 - 3183 2008 ;
- 39 . Gregory RI , Yan KP , Amuthan G , Chendrimada T , Doratotaj B , Cooch N , Shiekhattar R . The Microprocessor complex mediates the genesis of microRNAs . *Nature* . 432 : 235 - 240 2004 ;
- 40 . Guil S , Caceres JF . The multifunctional RNA-binding protein hnRNP A1 is required for processing of miR-18a . *Nat Struct Mol Biol* . 14 : 591 - 6 2007 ;
- 41 . Fukuda T , Yamagata K , Fujiyama S , Matsumoto T , Koshida I , Yoshimura K , Mihara M , Naitou M , Endoh H , Nakamura T , Akimoto C , Yamamoto Y , Katagiri T , Foulds C , Takezawa S , Kitagawa H , Takeyama K , O'Malley BW , Kato S . DEAD-box RNA helicase subunits of the Drosha complex are required for processing of rRNA and a subset of microRNAs . *Nat Cell Biol* . 9 : 604 - 11 2007 ;
- 42 . Lee Y , Ahn C , Han J , Choi H , Kim J , Yim J , Lee J , Provost P , Rådmark O , Kim S , Kim VN . The nuclear RNase III Drosha initiates microRNA processing . *Nature* . 425 : 415 - 419 2003 ;
- 43 . Han J , Lee Y , Yeom KH , Nam JW , Heo I , Rhee JK , Sohn SY , Cho Y , Zhang BT , Kim VN . Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex . *Cell* . 125 : 887 - 901 2006 ;
- 44 . Okamura K , Hagen JW , Duan H , Tyler DM , Lai EC . The mirtron pathway generates microRNA-class regulatory RNAs in Drosophila . *Cell* . 130 : 89 - 100 2007 ;
- 45 . Ruby JG , Jan CH , Bartel DP . Intronic microRNA precursors that bypass Drosha processing . *Nature* . 448 : 83 - 86 2007 ;
- 46 . Berezikov E , Chung WJ , Willis J , Cuppen E , Lai EC . Mammalian mirtron genes . *Mol Cell* . 28 : 328 - 336 2007 ;
- 47 . Lund E , Güttinger S , Calado A , Dahlberg JE , Kutay U . Nuclear export of microRNA precursors . *Science* . 303 : 95 - 98 2004 ;
- 48 . Yi R , Doehle BP , Qin Y , Macara IG , Cullen BR . Overexpression of exportin 5 enhances RNA interference mediated by short hairpin RNAs and microRNAs . *RNA* . 11 : 220 - 226 2005 ;
- 49 . Brownawell AM , Macara IG . Exportin-5, a novel karyopherin, mediates nuclear export of double-stranded RNA binding proteins . *J Cell Biol* . 156 : 53 - 64 2002 ;
- 50 . Gwizdek C , Ossareh-Nazari B , Brownawell AM , Doglio A , Bertrand E , Macara IG , Dargemont C . Exportin-5 mediates nuclear export of minihelix-containing RNAs . *J Biol Chem* . 278 : 5505 - 5508 2003 ;
- 51 . Zeng Y , Cullen BR . Structural requirements for pre-microRNA binding and nuclear export by Exportin 5 . *Nucleic Acids Res* . 32 : 4776 - 4785 2004 ;
- 52 . Kehlenbach RH , Dickmanns A , Kehlenbach A , Guan T , Gerace L . A role for RanBP1 in the release of CRM1 from the nuclear pore complex in a terminal step of nuclear export . *J Cell Biol* . 145 : 645 - 657 1999 ;
- 53 . Bernstein E , Caudy AA , Hammond SM , Hannon GJ . Role for a bidentate ribonuclease in the initiation step of RNA interference . *Nature* . 409 : 363 - 366 2001 ;
- 54 . Hutvagner G , McLachlan J , Pasquinelli AE , Bálint E , Tuschl T , Zamore PD . A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA . *Science* . 293 : 834 - 838 2001 ;
- 55 . Ketting RF , Fischer SE , Bernstein E , Sijen T , Hannon GJ , Plasterk RH . Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in *C. elegans* . *Genes Dev* . 15 : 2654 - 2659 2001 ;
- 56 . Song JJ , Liu J , Tolia NH , Schneiderman J , Smith SK , Martienssen RA , Hannon GJ , Joshua-Tor L . The crystal structure of the Argonaute2 PAZ domain reveals an RNA binding motif in RNAi effector complexes . *Nat Struct Mol Biol* . 10 : 1026 - 1032 2003 ;
- 57 . Ma JB , Ye K , Patel DJ . Structural basis for overhang-specific small interfering RNA recognition by the PAZ domain . *Nature* . 429 : 318 - 322 2004 ;
- 58 . Zhang H , Kolb FA , Brondani V , Billy E , Filipowicz W . Human Dicer preferentially cleaves dsRNAs at their termini without a requirement for ATP . *EMBO J* . 21 : 5875 - 5885 2002 ;
- 59 . Zhang H , Kolb FA , Jaskiewicz L , Westhof E , Filipowicz W . Single processing center models for human Dicer and bacterial RNase III . *Cell* . 118 : 57 - 68 2004 ;
- 60 . Chendrimada TP , Gregory RI , Kumaraswamy E , Norman J , Cooch N , Nishikura K , Shiekhattar R . TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing . *Nature* . 436 : 740 - 744 2005 ;
- 61 . Gregory RI , Chendrimada TP , Cooch N , Shiekhattar R . Human RISC couples microRNA biogenesis and posttranscriptional gene silencing . *Cell* . 123 : 631 - 640 2005 ;
- 62 . Maniataki E , Mourelatos Z . A human, ATP-independent, RISC assembly machine fueled by pre-miRNA . *Genes Dev* . 19 : 2979 - 2990 2005 ;
- 63 . MacRae IJ , Ma E , Zhou M , Robinson CV , Doudna JA . In vitro reconstitution of the human RISC-loading complex . *Proc Natl Acad Sci U S A* . 105 : 512 - 517 2008 ;
- 64 . Cosentino GP , Venkatesan S , Serluca FC , Green SR , Mathews MB , Sonenberg N . Double-stranded-RNA-dependent protein kinase and TAR RNA-binding protein form homo- and heterodimers in vivo . *Proc Natl Acad Sci U S A* . 92 : 9445 - 9449 1995 ;
- 65 . Kawamata T , Seitz H , Tomari Y . Structural determinants of miRNAs for RISC loading and slicer-independent unwinding . *Nat Struct Mol Biol* . 16 : 953 - 960 2009 ;
- 66 . Yoda M , Kawamata T , Paroo Z , Ye X , Iwasaki S , Liu Q , Tomari Y . ATP-dependent human RISC assembly pathways . *Nat Struct Mol Biol* . 17 : 17 - 23 2010 ;
- 67 . Mourelatos Z , Dostie J , Paushkin S , Sharma A , Charroux B , Abel L , Rappsilber J , Mann M , Dreyfuss G . miRNPs: a novel class of ribonucleoproteins containing numerous microRNAs . *Genes Dev* . 16 : 720 - 728 2002 ;
- 68 . Okamura K , Phillips MD , Tyler DM , Duan H , Chou Y-t , Lai EC . The regulatory activity of microRNA\* species has substantial influence on microRNA and 3' UTR evolution . *Nat Struct Mol Biol* . 15 : 354 - 363 2008 ;
- 69 . Schwarz DS , Hutvagner G , Du T , Xu Z , Aronin N , Zamore PD . Asymmetry in the assembly of the RNAi enzyme complex . *Cell* . 115 : 199 - 208 2003 ;
- 70 . Khvorovova A , Reynolds A , Jayasena SD . Functional siRNAs and miRNAs exhibit strand bias . *Cell* . 115 : 209 - 216 2003 ;
- 71 . Chu C-y , Rana TM . Translation repression in human cells by microRNA-induced gene silencing requires RCK/p54 . *PLoS Biol* . 4 : e210 - e210 2006 ;
- 72 . Wang B , Li S , Qi HH , Chowdhury D , Shi Y , Novina CD . Distinct passenger strand and mRNA cleavage activities of human Argonaute proteins . *Nat Struct Mol Biol* . 16 : 1259 - 1266 2009 ;
- 73 . Rand TA , Petersen S , Du F , Wang X . Argonaute2 cleaves the anti-guide strand of siRNA during RISC activation . *Cell* . 123 : 621 - 629 2005 ;
- 74 . Leuschner PJF , Ameres SL , Kueng S , Martinez J . Cleavage of the siRNA passenger strand during RISC assembly in human cells . *EMBO Rep* . 7 : 314 - 320 2006 ;
- 75 . Matranga C , Tomari Y , Shin C , Bartel DP , Zamore PD . Passenger-strand cleavage facilitates assembly of siRNA into Ago2-containing RNAi enzyme complexes . *Cell* . 123 : 607 - 620 2005 ;
- 76 . Shin C . Cleavage of the star strand facilitates assembly of some microRNAs into Ago2-containing silencing complexes in mammals . *Mol Cells* . 26 : 308 - 313 2008 ;

- 77 . Diederichs S , Haber DA . Dual role for argonautes in microRNA processing and posttranscriptional regulation of microRNA expression . *Cell* . 131 : 1097 - 1108 2007 ;
- 78 . Yuan YR , Pei Y , Ma JB , Kuryavyy V , Zhadina M , Meister G , Chen HY , Dauter Z , Tuschl T , Patel DJ . Crystal structure of A. aeolicus argonaute, a site-specific DNA-guided endoribonuclease, provides insights into RISC-mediated mRNA cleavage . *Mol Cell* . 19 : 405 - 419 2005 ;
- 79 . Lingel A , Simon B , Izaurralde E , Sattler M . Nucleic acid 3'-end recognition by the Argonaute2 PAZ domain . *Nat Struct Mol Biol* . 11 : 576 - 577 2004 ;
- 80 . Boland A , Tritschler F , Heimstädt S , Izaurralde E , Weichenrieder O . Crystal structure and ligand binding of the MID domain of a eukaryotic Argonaute protein . *EMBO Rep* . 11 : 522 - 527 2010 ;
- 81 . Djuranovic S , Zinchenko MK , Hur JK , Nahvi A , Brunelle JL , Rogers EJ , Green R . Allosteric regulation of Argonaute proteins by miRNAs . *Nat Struct Mol Biol* . 17 : 144 - 150 2010 ;
- 82 . Song JJ , Smith SK , Hannon GJ , Joshua-Tor L . Crystal structure of Argonaute and its implications for RISC slicer activity . *Science* . 305 : 1434 - 1437 2004 ;
- 83 . Okamura K , Ishizuka A , Siomi H , Siomi MC . Distinct roles for Argonaute proteins in small RNA-directed RNA cleavage pathways . *Genes Dev* . 18 : 1655 - 1666 2004 ;
- 84 . Liu J , Carmell MA , Rivas FV , Marsden CG , Thomson JM , Song JJ , Hammond SM , Joshua-Tor L , Hannon GJ . Argonaute2 is the catalytic engine of mammalian RNAi . *Science* . 305 : 1437 - 1441 2004 ;
- 85 . Meister G , Landthaler M , Patkaniowska A , Dorsett Y , Teng G , Tuschl T . Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs . *Mol Cell* . 15 : 185 - 197 2004 ;
- 86 . Rhoades MW , Reinhart BJ , Lim LP , Burge CB , Bartel B , Bartel DP . Prediction of plant microRNA targets . *Cell* . 110 : 513 - 520 2002 ;
- 87 . Jones-Rhoades MW , Bartel DP , Bartel B . MicroRNAs and their regulatory roles in plants . *Annu Rev Plant Biol* . 57 : 19 - 53 2006 ;
- 88 . Lewis BP , Shih IH , Jones-Rhoades MW , Bartel DP , Burge CB . Prediction of mammalian microRNA targets . *Cell* . 115 : 787 - 798 2003 ;
- 89 . Doench JG , Sharp PA . Specificity of microRNA target selection in translational repression . *Genes Dev* . 18 : 504 - 511 2004 ;
- 90 . Brennecke J , Stark A , Russell RB , Cohen SM . Principles of microRNA-target recognition . *PLoS Biol* . 3 : e85 - e85 2005 ;
- 91 . Lewis BP , Burge CB , Bartel DP . Conserved seed pairing, often flanked by adenines, indicates that thousands of human genes are microRNA targets . *Cell* . 120 : 15 - 20 2005 ;
- 92 . Elbashir SM , Martinez J , Patkaniowska A , Lendeckel W , Tuschl T . Functional anatomy of siRNAs for mediating efficient RNAi in *Drosophila melanogaster* embryo lysate . *EMBO J* . 20 : 6877 - 88 2001 ;
- 93 . Grimson A , Farh KKH , Johnston WK , Garrett-Engle P , Lim LP , Bartel DP . MicroRNA targeting specificity in mammals: determinants beyond seed pairing . *Mol Cell* . 27 : 91 - 105 2007 ;
- 94 . Filipowicz W , Bhattacharyya SN , Sonenberg N . Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? . *Nat Rev Genet* . 9 : 102 - 114 2008 ;
- 95 . Forman JJ , Collier HA . The code within the code: microRNAs target coding regions . *Cell Cycle* . 9 : 1533 - 41 2010 ;
- 96 . Lytle JR , Yario TA , Steitz JA . Target mRNAs are repressed as efficiently by microRNA-binding sites in the 5' UTR as in the 3' UTR . *Proc Natl Acad Sci U S A* . 104 : 9667 - 72 2007 ;
- 97 . Bartel DP . MicroRNAs: target recognition and regulatory functions . *Cell* . 136 : 215 - 233 2009 ;
- 98 . Friedman RC , Farh KKH , Burge CB , Bartel DP . Most mammalian mRNAs are conserved targets of microRNAs . *Genome Res* . 19 : 92 - 105 2009 ;
- 99 . Krek A , Grün D , Poy MN , Wolf R , Rosenberg L , Epstein EJ , MacMenamin P , da Piedade I , Gunsalus KC , Stoffel M , Rajewsky N . Combinatorial microRNA target predictions . *Nat Genet* . 37 : 495 - 500 2005 ;
- 100 . John B , Enright AJ , Aravin A , Tuschl T , Sander C , Marks DS . Human MicroRNA targets . *PLoS Biol* . 2 : e363 - e363 2004 ;
- 101 . Betel D , Wilson M , Gabow A , Marks DS , Sander C . The microRNA.org resource: targets and expression . *Nucleic Acids Res* . 36 : D149-153 - D149-153 2008 ;
- 102 . Kertesz M , Iovino N , Unnerstall U , Gaul U , Segal E . The role of site accessibility in microRNA target recognition . *Nat Genet* . 39 : 1278 - 1284 2007 ;
- 103 . Gaidatzis D , van Nimwegen E , Hausser J , Zavolan M . Inference of miRNA targets using evolutionary conservation and pathway analysis . *BMC Bioinformatics* . 8 : 69 - 69 2007 ;
- 104 . Kiriakidou M , Nelson PT , Kouranov A , Fitziev P , Bouyioukos C , Mourelatos Z , Hatzigeorgiou A . A combined computational-experimental approach predicts human microRNA targets . *Genes Dev* . 18 : 1165 - 1178 2004 ;
- 105 . Sethupathy P , Megraw M , Hatzigeorgiou AG . A guide through present computational approaches for the identification of mammalian microRNA targets . *Nat Meth* . 3 : 881 - 886 2006 ;
- 106 . Lam LT , Lu X , Zhang H , Lesniewski R , Rosenberg S , Semizarov D . A microRNA screen to identify modulators of sensitivity to BCL2 inhibitor ABT-263 (navitoclax) . *Mol Cancer Ther* . 9 : 2943 - 50 2010 ;
- 107 . Sethupathy P , Corda B , Hatzigeorgiou AG . TarBase: A comprehensive database of experimentally supported animal microRNA targets . *RNA* . 12 : 192 - 197 2006 ;
- 108 . Papadopoulos GL , Reczko M , Simossis VA , Sethupathy P , Hatzigeorgiou AG . The database of experimentally supported targets: a functional update of TarBase . *Nucleic Acids Res* . 37 : D155-158 - D155-158 2009 ;
- 109 . Pestova TV , Kolupaeva G , Lomakin IB , Pilipenko EV , Shatsky IN , Agol VI , Hellen CU . Molecular mechanisms of translation initiation in eukaryotes . *Proc Natl Acad Sci U S A* . 98 : 7029 - 7036 2001 ;
- 110 . Gebauer F , Hentze MW . Molecular mechanisms of translational control . *Nat Rev Mol Cell Biol* . 5 : 827 - 835 2004 ;
- 111 . Kean KM . The role of mRNA 5'-noncoding and 3'-end sequences on 40S ribosomal subunit recruitment, and how RNA viruses successfully compete with cellular mRNAs to ensure their own protein synthesis . *Biol Cell* . 95 : 129 - 139 2003 ;
- 112 . Humphreys DT , Westman BJ , Martin DIK , Preiss T . MicroRNAs control translation initiation by inhibiting eukaryotic initiation factor 4E/cap and poly(A) tail function . *Proc Natl Acad Sci U S A* . 102 : 16961 - 16966 2005 ;
- 113 . Pillai RS , Bhattacharyya SN , Artus CG , Zoller T , Cougot N , Basyuk E , Bertrand E , Filipowicz W . Inhibition of translational initiation by Let-7 MicroRNA in human cells . *Science* . 309 : 1573 - 1576 2005 ;
- 114 . Kiriakidou M , Tan GS , Lamprinak S , De Planell-Saguer M , Nelson PT , Mourelatos Z . An mRNA m7G cap binding-like motif within human Ago2 represses translation . *Cell* . 129 : 1141 - 1151 2007 ;
- 115 . Chendrimada TP , Finn KJ , Ji X , Baillat D , Gregory RI , Liebhaber SA , Pasquinelli AE , Shiekhattar R . MicroRNA silencing through RISC recruitment of eIF6 . *Nature* . 447 : 823 - 828 2007 ;
- 116 . Olsen PH , Ambros V . The lin-4 regulatory RNA controls developmental timing in *Caenorhabditis elegans* by blocking LIN-14 protein synthesis after the initiation of translation . *Dev Biol* . 216 : 671 - 680 1999 ;
- 117 . Slegger K , Tang L , Moss EG . Two genetic circuits repress the *Caenorhabditis elegans* heterochronic gene lin-28 after translation initiation . *Dev Biol* . 243 : 215 - 225 2002 ;
- 118 . Petersen CP , Bordeleau ME , Pelletier J , Sharp PA . Short RNAs repress translation after initiation in mammalian cells . *Mol Cell* . 21 : 533 - 542 2006 ;
- 119 . Maroney PA , Yu Y , Fisher J , Nilsen TW . Evidence that microRNAs are associated with translating messenger RNAs in human cells . *Nat Struct Mol Biol* . 13 : 1102 - 1107 2006 ;
- 120 . Nottrott S , Simard MJ , Richter JD . Human let-7a miRNA blocks protein production on actively translating polyribosomes . *Nat Struct Mol Biol* . 13 : 1108 - 1114 2006 ;
- 121 . Bagga S , Bracht J , Hunter S , Massirer K , Holtz J , Eachus R , Pasquinelli AE . Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation . *Cell* . 122 : 553 - 563 2005 ;
- 122 . Wu L , Belasco JG . Micro-RNA regulation of the mammalian lin-28 gene during neuronal differentiation of embryonal carcinoma cells . *Mol Cell Biol* . 25 : 9198 - 9208 2005 ;
- 123 . Guo H , Ingolia NT , Weissman JS , Bartel DP . Mammalian microRNAs predominantly act to decrease target mRNA levels . *Nature* . 466 : 835 - 40 2010 ;

- 124 . Wu L , Fan J , Belasco JG . MicroRNAs direct rapid deadenylation of mRNA . *Proc Natl Acad Sci U S A* . 103 : 4034 - 4039 2006 ;
- 125 . Parker R , Song H . The enzymes and control of eukaryotic mRNA turnover . *Nat Struct Mol Biol* . 11 : 121 - 127 2004 ;
- 126 . Eulalio A , Behm-Ansmant I , Izaurralde E . P bodies: at the crossroads of post-transcriptional pathways . *Nat Rev Mol Cell Biol* . 8 : 9 - 22 2007 ;
- 127 . Ingelfinger D , Arndt-Jovin DJ , Lührmann R , Achsel T . The human LSM1-7 proteins colocalize with the mRNA-degrading enzymes Dcp1/2 and Xrn1 in distinct cytoplasmic foci . *RNA* . 8 : 1489 - 1501 2002 ;
- 128 . Sheth U , Parker R . Decapping and decay of messenger RNA occur in cytoplasmic processing bodies . *Science* . 300 : 805 - 808 2003 ;
- 129 . Brengues M , Teixeira D , Parker R . Movement of eukaryotic mRNAs between polysomes and cytoplasmic processing bodies . *Science* . 310 : 486 - 489 2005 ;
- 130 . Eystathiou T , Chan EKL , Tenenbaum SA , Keene JD , Griffith K , Fritzler MJ . A phosphorylated cytoplasmic autoantigen, GW182, associates with a unique population of human mRNAs within novel cytoplasmic speckles . *Mol Biol Cell* . 13 : 1338 - 1351 2002 ;
- 131 . Liu J , Rivas FV , Wohlschlegel J , Yates JR , Parker R , Hannon GJ . A role for the P-body component GW182 in microRNA function . *Nat Cell Biol* . 7 : 1261 - 1266 2005 ;
- 132 . Kedersha N , Anderson P . Stress granules: sites of mRNA triage that regulate mRNA stability and translatability . *Biochem Soc Trans* . 30 : 963 - 969 2002 ;
- 133 . Cougot N , Babajko S , Séraphin B . Cytoplasmic foci are sites of mRNA decay in human cells . *J Cell Biol* . 165 : 31 - 40 2004 ;
- 134 . Kedersha N , Stoecklin G , Ayodele M , Yacono P , Lykke-Andersen J , Fritzler MJ , Scheuner D , Kaufman RJ , Golan DE , Anderson P . Stress granules and processing bodies are dynamically linked sites of mRNP remodeling . *J Cell Biol* . 169 : 871 - 884 2005 ;
- 135 . Vasudevan S , Steitz JA . AU-rich-element-mediated upregulation of translation by FXR1 and Argonaute 2 . *Cell* . 128 : 1105 - 1118 2007 ;
- 136 . Eiring AM , Harb JG , Neviani P , Garton C , Oaks JJ , Spizzo R , Liu S , Schwind S , Santhanam R , Hickey CJ , Becker H , Chandler JC , Andino R , Cortes J , Hokland P , Huettner CS , Bhatia R , Roy DC , Liebhaber SA , Caligiuri MA , Marcucci G , Garzon R , Croce CM , Calin GA , Perrotti D . miR-328 functions as an RNA decoy to modulate hnRNP E2 regulation of mRNA translation in leukemic blasts . *Cell* . 140 : 652 - 65 2010 ;
- 137 . Brennecke J , Hipfner DR , Stark A , Russell RB , Cohen SM . bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in *Drosophila* . *Cell* . 113 : 25 - 36 2003 ;
- 138 . Chen CZ , Li L , Lodish HF , Bartel DP . MicroRNAs modulate hematopoietic lineage differentiation . *Science* . 303 : 83 - 86 2004 ;
- 139 . Harfe BD . MicroRNAs in vertebrate development . *Curr Opin Genet Dev* . 15 : 410 - 415 2005 ;
- 140 . Pasquinelli AE , Hunter S , Bracht J . MicroRNAs: a developing story . *Curr Opin Genet Dev* . 15 : 200 - 205 2005 ;
- 141 . Lu J , Getz G , Miska EA , Alvarez-Saavedra E , Lamb J , Peck D , Sweet-Cordero A , Ebert BL , Mak RH , Ferrando AA , Downing JR , Jacks T , Horvitz HR , Golub TR . MicroRNA expression profiles classify human cancers . *Nature* . 435 : 834 - 838 2005 ;
- 142 . Volinia S , Calin GA , Liu CG , Ambs S , Cimmino A , Petrocca F , Visone R , Iorio M , Roldo C , Ferracin M , Prueitt RL , Yanaihara N , Lanza G , Scarpa A , Vecchione A , Negrini M , Harris CC , Croce CM . A microRNA expression signature of human solid tumors defines cancer gene targets . *Proc Natl Acad Sci U S A* . 103 : 2257 - 61 2006 ;
- 143 . Wang Y , Lee CGL . MicroRNA and cancer--focus on apoptosis . *J Cell Mol Med* . 13 : 12 - 23 2009 ;
- 144 . Lages E , Guttin A , El Atifi M , Ramus C , Ipas H , Dupre I , Rolland D , Salon C , Godfraind C , deFraipont F , Dhobb M , Pelletier L , Wion D , Gay E , Berger F , Issartel JP . MicroRNA and target protein patterns reveal physiopathological features of glioma subtypes . *PLoS One* . 6 : e20600 - 2011 ;
- 145 . Kitano M , Rahbari R , Patterson EE , Xiong Y , Prasad NB , Wang Y , Zeiger MA , Kebebew E . Expression Profiling of Difficult-to-diagnose Thyroid Histologic Subtypes Shows Distinct Expression Profiles and Identify Candidate Diagnostic microRNAs . *Ann Surg Oncol* . 18 : 3443 - 52 2011 ;
- 146 . Youssef YM , White NM , Grigull J , Krizova A , Samy C , Mejia-Guerrero S , Evans A , Yousef GM . Accurate molecular classification of kidney cancer subtypes using microRNA signature . *Eur Urol* . 59 : 721 - 30 2011 ;
- 147 . Merritt WM , Lin YG , Han LY , Kamat AA , Spanuth WA , Schmandt R , Urbauer D , Pennacchio LA , Cheng JF , Nick AM , Deavers MT , Mourad-Zeidan A , Wang H , Mueller P , Lenburg ME , Gray JW , Mok S , Birrer MJ , Lopez-Berestein G , Coleman RL , Bar-Eli M , Sood AK . Dicer, Drosha, and outcomes in patients with ovarian cancer . *N Engl J Med* . 359 : 2641 - 2650 2008 ;
- 148 . Sand M , Gambichler T , Skrygan M , Sand D , Scola N , Altmeyer P , Bechara FG . Expression levels of the microRNA processing enzymes Drosha and dicer in epithelial skin cancer . *Cancer Investig* . 28 : 649 - 653 2010 ;
- 149 . Sugito N , Ishiguro H , Kuwabara Y , Kimura M , Mitsui A , Kurehara H , Ando T , Mori R , Takashima N , Ogawa R , Fujii Y . RNASEN regulates cell proliferation and affects survival in esophageal cancer patients . *Clin Cancer Res* . 12 : 7322 - 7328 2006 ;
- 150 . Melo SA , Moutinho C , Ropero S , Calin GA , Rossi S , Spizzo R , Fernandez AF , Davalos V , Villanueva A , Montoya G , Yamamoto H , Schwartz S Jr , Esteller M . A genetic defect in exportin-5 traps precursor microRNAs in the nucleus of cancer cells . *Cancer Cell* . 18 : 303 - 15 2010 ;
- 151 . Chiosea S , Jelezcova E , Chandran U , Acquafondata M , McHale T , Sobol RW , Dhir R . Up-regulation of dicer, a component of the MicroRNA machinery, in prostate adenocarcinoma . *Am J Pathol* . 169 : 1812 - 1820 2006 ;
- 152 . Chiosea S , Jelezcova E , Chandran U , Luo J , Mantha G , Sobol RW , Dacic S . Overexpression of Dicer in precursor lesions of lung adenocarcinoma . *Cancer Res* . 67 : 2345 - 2350 2007 ;
- 153 . Karube Y , Tanaka H , Osada H , Tomida S , Tatematsu Y , Yanagisawa K , Yatabe Y , Takamizawa J , Miyoshi S , Mitsudomi T , Takahashi T . Reduced expression of Dicer associated with poor prognosis in lung cancer patients . *Cancer Sci* . 96 : 111 - 115 2005 ;
- 154 . Melo SA , Ropero S , Moutinho C , Aaltonen LA , Yamamoto H , Calin GA , Rossi S , Fernandez AF , Carneiro F , Oliveira C , Ferreira B , Liu CG , Villanueva A , Capella G , Schwartz S Jr , Shiekhhattar R , Esteller M . A TARBP2 mutation in human cancer impairs microRNA processing and DICER1 function . *Nat Genet* . 41 : 365 - 70 2009 ;
- 155 . Boominathan L . The tumor suppressors p53, p63, and p73 are regulators of microRNA processing complex . *PLoS One* . 5 : e10615 - e10615 2010 ;
- 156 . Calin GA , Sevignani C , Dumitru CD , Hyslop T , Noch E , Yendamuri S , Shimizu M , Rattan S , Bullrich F , Negrini M , Croce CM . Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers . *Proc Natl Acad Sci U S A* . 101 : 2999 - 3004 2004 ;
- 157 . Zhang L , Coukos G . MicroRNAs: a new insight into cancer genome . *Cell Cycle* . 5 : 2216 - 2219 2006 ;
- 158 . Bousquet M , Quelen C , Rosati R , Mas V , Mansat-De , Starza R La , Bastard C , Lippert E , Talmant P , Lafage-Pochitaloff M , Leroux D , Gervais C , Viguié F , Lai J-L , Terre C , Beverlo B , Sambani C , Hagemeyer A , Marynen P , Delsol G , Dastugue N , Mecucci C , Brousset P . Myeloid cell differentiation arrest by miR-125b-1 in myelodysplastic syndrome and acute myeloid leukemia with the t(2;11)(p21;q23) translocation . *J Exp Med* . 205 : 2499 - 2506 2008 ;
- 159 . Saito-Ohara F , Imoto I , Inoue J , Hosoi H , Nakagawara A , Sugimoto T , Inazawa J . PPM1D is a potential target for 17q gain in neuroblastoma . *Cancer Res* . 63 : 1876 - 1883 2003 ;
- 160 . Calin GA , Dumitru CD , Shimizu M , Bichi R , Zupo S , Noch E , Aldler H , Rattan S , Keating M , Rai K , Rassenti L , Kipps T , Negrini M , Bullrich F , Croce CM . Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia . *Proc Natl Acad Sci U S A* . 99 : 15524 - 15529 2002 ;
- 161 . Saunders MA , Liang H , Li WH . Human polymorphism at microRNAs and microRNA target sites . *Proc Natl Acad Sci U S A* . 104 : 3300 - 3305 2007 ;
- 162 . Jazdzewski K , Murray EL , Franssila K , Jarzab B , Schoenberg DR , de la Chapelle A . Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma . *Proc Natl Acad Sci U S A* . 105 : 7269 - 7274 2008 ;
- 163 . Xu B , Feng NH , Li PC , Tao J , Wu D , Zhang ZD , Tong N , Wang JF , Song NH , Zhang W , Hua LX , Wu HF . A functional polymorphism in Pre-miR-146a gene is associated with prostate cancer risk and mature miR-146a expression in vivo . *Prostate* . 70 : 467 - 472 2010 ;
- 164 . Duan R , Pak C , Jin P . Single nucleotide polymorphism associated with mature miR-125a alters the processing of pri-miRNA . *Hum Mol Genet* . 16 : 1124 - 1131 2007 ;
- 165 . Li W , Duan R , Kooy F , Sherman SL , Zhou W , Jin P . Germline mutation of microRNA-125a is associated with breast cancer . *J Med Genet* . 46 : 358 - 360 2009 ;
- 166 . Hu Z , Chen J , Tian T , Zhou X , Gu H , Xu L , Zeng Y , Miao R , Jin G , Ma H , Chen Y , Shen H . Genetic variants of miRNA sequences and non-small cell lung cancer survival . *J Clin Invest* . 118 : 2600 - 2608 2008 ;



- 167 . Calin GA , Ferracin M , Cimmino A , Di Leva G , Shimizu M , Wojcik SE , Iorio MV , Visone R , Sever NI , Fabbri M , Iuliano R , Palumbo T , Pichiorri F , Roldo C , Garzon R , Sevignani C , Rassenti L , Alder H , Volinia S , Liu CG , Kipps TJ , Negrini M , Croce CM . A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia . *N Engl J Med* . 353 : 1793 - 801 2005 ;
- 168 . Kotani A , Ha D , Schotte D , den Boer ML , Armstrong SA , Lodish HF . A novel mutation in the miR-128b gene reduces miRNA processing and leads to glucocorticoid resistance of MLL-AF4 acute lymphocytic leukemia cells . *Cell Cycle* . 9 : 1037 - 42 2010 ;
- 169 . Ohler U , Yekta S , Lim LP , Bartel DP , Burge CB . Patterns of flanking sequence conservation and a characteristic upstream motif for microRNA gene identification . *RNA* . 10 : 1309 - 1322 2004 ;
- 170 . Xi Y , Shalgi R , Fodstad O , Pilpel Y , Ju J . Differentially regulated micro-RNAs and actively translated messenger RNA transcripts by tumor suppressor p53 in colon cancer . *Clin Cancer Res* . 12 : 2014 - 2024 2006 ;
- 171 . Chang TC , Wentzel EA , Kent OA , Ramachandran K , Mullendore M , Lee KH , Feldmann G , Yamakuchi M , Ferlito M , Lowenstein CJ , Arking DE , Beer MA , Maitra A , Mendell JT . Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis . *Mol Cell* . 26 : 745 - 752 2007 ;
- 172 . Raver-Shapira N , Marciano E , Meiri E , Spector Y , Rosenfeld N , Moskovits N , Bentwich Z , Oren M . Transcriptional activation of miR-34a contributes to p53-mediated apoptosis . *Mol Cell* . 26 : 731 - 743 2007 ;
- 173 . Tarasov V , Jung P , Verdoodt B , Lodygin D , Epanchintsev A , Menssen A , Meister G , Hermeking H . Differential regulation of microRNAs by p53 revealed by massively parallel sequencing: miR-34a is a p53 target that induces apoptosis and G1-arrest . *Cell Cycle* . 6 : 1586 - 1593 2007 ;
- 174 . Brosh R , Shalgi R , Liran A , Landan G , Korotayev K , Nguyen GH , Enerly E , Johnsen H , Buganim Y , Solomon H , Goldstein I , Madar S , Goldfinger N , Børresen-Dale AL , Ginsberg D , Harris CC , Pilpel Y , Oren M , Rotter V . p53-Repressed miRNAs are involved with E2F in a feed-forward loop promoting proliferation . *Mol Syst Biol* . 4 : 229 - 229 2008 ;
- 175 . Bueno MJ , Gómez de Cedrón M , Laresgoiti U , Fernández-Piqueras J , Zubiaga AM , Malumbres M . Multiple E2F-induced microRNAs prevent replicative stress in response to mitogenic signaling . *Mol Cell Biol* . 30 : 2983 - 2995 2010 ;
- 176 . O'Donnell KA , Wentzel EA , Zeller KI , Dang CV , Mendell JT . c-Myc-regulated microRNAs modulate E2F1 expression . *Nature* . 435 : 839 - 843 2005 ;
- 177 . Chang TC , Yu D , Lee YS , Wentzel EA , Arking DE , West KM , Dang CV , Thomas-Tikhonenko A , Mendell JT . Widespread microRNA repression by Myc contributes to tumorigenesis . *Nat Genet* . 40 : 43 - 50 2008 ;
- 178 . Tate PH , Bird AP . Effects of DNA methylation on DNA-binding proteins and gene expression . *Curr Opin Genet Dev* . 3 : 226 - 231 1993 ;
- 179 . Saito Y , Jones PA . Epigenetic activation of tumor suppressor microRNAs in human cancer cells . *Cell Cycle* . 5 : 2220 - 2222 2006 ;
- 180 . Saito Y , Liang G , Egger G , Friedman JM , Chuang JC , Coetzee GA , Jones PA . Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells . *Cancer Cell* . 9 : 435 - 443 2006 ;
- 181 . Lujambio A , Ropero S , Ballestar E , Fraga MF , Cerrato C , Setién F , Casado S , Suarez-Gauthier A , Sanchez-Cespedes M , Git A , Gitt A , Spiteri I , Das PP , Caldas C , Miska E , Esteller M . Genetic unmasking of an epigenetically silenced microRNA in human cancer cells . *Cancer Res* . 67 : 1424 - 1429 2007 ;
- 182 . Lodygin D , Tarasov V , Epanchintsev A , Berking C , Knyazeva T , Körner H , Knyazev P , Diebold J , Hermeking H . Inactivation of miR-34a by aberrant CpG methylation in multiple types of cancer . *Cell Cycle* . 7 : 2591 - 2600 2008 ;
- 183 . Mi S , Lu J , Sun M , Li Z , Zhang H , Neilly MB , Wang Y , Qian Z , Jin J , Zhang Y , Bohlander SK , Le Beau MM , Larson RA , Golub TR , Rowley JD , Chen J . MicroRNA expression signatures accurately discriminate acute lymphoblastic leukemia from acute myeloid leukemia . *Proc Natl Acad Sci U S A* . 104 : 19971 - 19976 2007 ;
- 184 . Brueckner B , Stresmann C , Kuner R , Mund C , Musch T , Meister M , Sültmann H , Lyko F . The human let-7a-3 locus contains an epigenetically regulated microRNA gene with oncogenic function . *Cancer Res* . 67 : 1419 - 1423 2007 ;
- 185 . Gao Y , He Y , Ding J , Wu K , Hu B , Liu Y , Wu Y , Guo B , Shen Y , Landi D , Landi S , Zhou Y , Liu H . An insertion/deletion polymorphism at miRNA-122-binding site in the interleukin-1alpha 3' untranslated region confers risk for hepatocellular carcinoma . *Carcinogenesis* . 30 : 2064 - 2069 2009 ;
- 186 . Wiestner A , Tehrani M , Chiorazzi M , Wright G , Gibellini F , Nakayama K , Liu H , Rosenwald A , Muller-Hermelink HK , Ott G , Chan WC , Greiner TC , Weisenburger DD , Vose J , Armitage JO , Gascoyne RD , Connors JM , Campo E , Montserrat E , Bosch F , Smeland EB , Kvaloy S , Holte H , Delabie J , Fisher RI , Grogan TM , Miller TP , Wilson WH , Jaffe ES , Staudt LM . Point mutations and genomic deletions in CCND1 create stable truncated cyclin D1 mRNAs that are associated with increased proliferation rate and shorter survival . *Blood* . 109 : 4599 - 4606 2007 ;
- 187 . Chen RW , Bemis LT , Amato CM , Myint H , Tran H , Birks DK , Eckhardt SG , Robinson WA . Truncation in CCND1 mRNA alters miR-16-1 regulation in mantle cell lymphoma . *Blood* . 112 : 822 - 829 2008 ;
- 188 . Mayr C , Hemann MT , Bartel DP . Disrupting the pairing between let-7 and Hmga2 enhances oncogenic transformation . *Science* . 315 : 1576 - 1579 2007 ;
- 189 . Sarhadi VK , Wikman H , Salmenkivi K , Kuosma E , Sioris T , Salo J , Karjalainen A , Knuutila S , Anttila S . Increased expression of high mobility group A proteins in lung cancer . *J Pathol* . 209 : 206 - 212 2006 ;
- 190 . Meyer B , Loeschke S , Schultze A , Weigel T , Sandkamp M , Goldmann T , Vollmer E , Bullerdiek J . HMGA2 overexpression in non-small cell lung cancer . *Mol Carcinog* . 46 : 503 - 511 2007 ;
- 191 . Di Cello F , Hillion J , Hristov A , Wood LJ , Mukherjee M , Schuldenfrei A , Kowalski J , Bhattacharya R , Ashfaq R , Resar LMS . HMGA2 participates in transformation in human lung cancer . *Mol Cancer Res* . 6 : 743 - 750 2008 ;
- 192 . Abe N , Watanabe T , Suzuki Y , Matsumoto N , Masaki T , Mori T , Sugiyama M , Chiappetta G , Fusco A , Atomi Y . An increased high-mobility group A2 expression level is associated with malignant phenotype in pancreatic exocrine tissue . *Br J Cancer* . 89 : 2104 - 2109 2003 ;
- 193 . To KK , Zhan Z , Litman T , Bates SE . Regulation of ABCG2 expression at the 3' untranslated region of its mRNA through modulation of transcript stability and protein translation by a putative microRNA in the S1 colon cancer cell line . *Mol Cell Biol* . 28 : 5147 - 61 2008 ;
- 194 . Yu Z , Li Z , Jolicœur N , Zhang L , Fortin Y , Wang E , Wu M , Shen SH . Aberrant allele frequencies of the SNPs located in microRNA target sites are potentially associated with human cancers . *Nucleic Acids Res* . 35 : 4535 - 4541 2007 ;
- 195 . He H , Jazdzewski K , Li W , Liyanarachchi S , Nagy R , Volinia S , Calin GA , Liu CG , Franssila K , Suster S , Kloos RT , Croce CM , de la Chapelle A . The role of microRNA genes in papillary thyroid carcinoma . *Proc Natl Acad Sci U S A* . 102 : 19075 - 19080 2005 ;
- 196 . Landi D , Gemignani F , Naccarati A , Pardini B , Vodicka P , Vodickova L , Novotny J , Försti A , Hemminki K , Canzian F , Landi S . Polymorphisms within micro-RNA-binding sites and risk of sporadic colorectal cancer . *Carcinogenesis* . 29 : 579 - 584 2008 ;
- 197 . Chin LJ , Ratner E , Leng S , Zhai R , Nallur S , Babar I , Muller RU , Straka E , Su L , Burki EA , Crowell RE , Patel R , Kulkarni T , Homer R , Zelterman D , Kidd KK , Zhu Y , Christiani DC , Belinsky SA , Slack FJ , Weidhaas JB . A SNP in a let-7 microRNA complementary site in the KRAS 3' untranslated region increases non-small cell lung cancer risk . *Cancer Res* . 68 : 8535 - 8540 2008 ;
- 198 . Paranjape T , Heneghan H , Lindner R , Keane FK , Hoffman A , Hollestelle A , Dorairaj J , Geyda K , Pelletier C , Nallur S , Martens JW , Hooning MJ , Kerin M , Zelterman D , Zhu Y , Tuck D , Harris L , Miller N , Slack F , Weidhaas J . A 3'-untranslated region KRAS variant and triple-negative breast cancer: a case-control and genetic analysis . *Lancet Oncol* . 12 : 377 - 86 2011 ;
- 199 . Brendle A , Lei H , Brandt A , Johansson R , Enquist K , Henriksson R , Hemminki K , Lenner P , Försti A . Polymorphisms in predicted microRNA-binding sites in integrin genes and breast cancer: ITGB4 as prognostic marker . *Carcinogenesis* . 29 : 1394 - 1399 2008 ;
- 200 . Voorhoeve PM , le Sage C , Schrier M , Gillis AJM , Stoop H , Nagel R , Liu YP , van Duijse J , Drost J , Griekspoor A , Zlotorynski E , Yabuta N , De Vita G , Nojima H , Looijenga LHJ , Agami R . A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors . *Cell* . 124 : 1169 - 1181 2006 ;
- 201 . Hayashita Y , Osada H , Tatematsu Y , Yamada H , Yanagisawa K , Tomida S , Yatabe Y , Kawahara K , Sekido Y , Takahashi T . A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation . *Cancer Res* . 65 : 9628 - 9632 2005 ;
- 202 . He L , Thomson JM , Hemann MT , Hernando-Monge E , Mu D , Goodson S , Powers S , Cordon-Cardo C , Lowe SW , Hannon GJ , Hammond SM . A microRNA polycistron as a potential human oncogene . *Nature* . 435 : 828 - 833 2005 ;
- 203 . Tam W , Hughes SH , Hayward WS , Besmer P . Avian bic, a gene isolated from a common retroviral site in avian leukosis virus-induced lymphomas that encodes a noncoding RNA, cooperates with c-myc in lymphomagenesis and erythroleukemogenesis . *J Virol* . 76 : 4275 - 4286 2002 ;

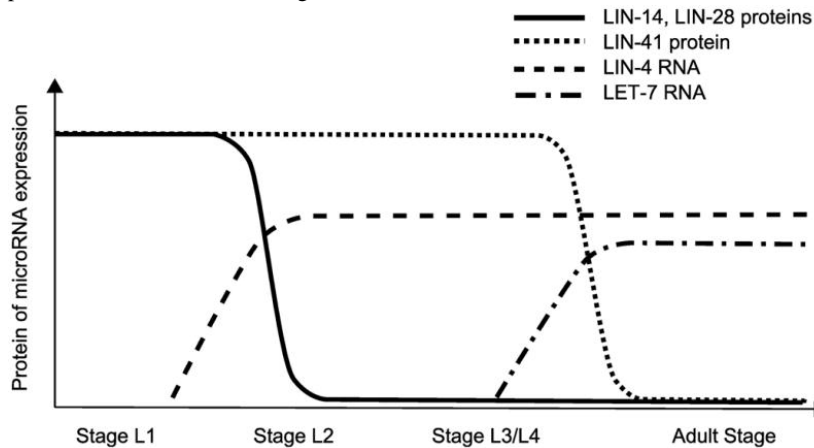
- 204 . Eis PS , Tam W , Sun L , Chadburn A , Li Z , Gomez MF , Lund E , Dahlberg JE . Accumulation of miR-155 and BIC RNA in human B cell lymphomas . *Proc Natl Acad Sci U S A* . 102 : 3627 - 3632 2005 ;
- 205 . Kluiver J , Poppema S , de Jong D , Blokzijl T , Harms G , Jacobs S , Kroesen BJ , van den Berg A . BIC and miR-155 are highly expressed in Hodgkin, primary mediastinal and diffuse large B cell lymphomas . *J Pathol* . 207 : 243 - 249 2005 ;
- 206 . Costinean S , Zanesi N , Pekarsky Y , Tili E , Volinia S , Heerema N , Croce CM . Pre-B cell proliferation and lymphoblastic leukemia/high-grade lymphoma in E(mu)-miR155 transgenic mice . *Proc Natl Acad Sci U S A* . 103 : 7024 - 7029 2006 ;
- 207 . Chen Y , Knösel T , Kristiansen G , Pietas A , Garber ME , Matsushashi S , Ozaki I , Petersen I . Loss of PDCD4 expression in human lung cancer correlates with tumour progression and prognosis . *J Pathol* . 200 : 640 - 646 2003 ;
- 208 . Mudduluru G , Medved F , Grobholz R , Jost C , Gruber A , Leupold JH , Post S , Jansen A , Colburn NH , Allgayer H . Loss of programmed cell death 4 expression marks adenoma-carcinoma transition, correlates inversely with phosphorylated protein kinase B, and is an independent prognostic factor in resected colorectal cancer . *Cancer* . 110 : 1697 - 1707 2007 ;
- 209 . Zhu S , Si ML , Wu H , Mo YY . MicroRNA-21 targets the tumor suppressor gene tropomyosin 1 (TPM1) . *J Biol Chem* . 282 : 14328 - 14336 2007 ;
- 210 . Asangani IA , Rasheed SAK , Nikolova DA , Leupold JH , Colburn NH , Post S , Allgayer H . MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdc4 and stimulates invasion, intravasation and metastasis in colorectal cancer . *Oncogene* . 27 : 2128 - 2136 2008 ;
- 211 . Iorio MV , Ferracin M , Liu CG , Veronese A , Spizzo R , Sabbioni S , Magri E , Pedriali M , Fabbri M , Campiglio M , Ménard S , Palazzo JP , Rosenberg A , Musiani P , Volinia S , Nenci I , Calin GA , Querzoli P , Negrini M , Croce CM . MicroRNA gene expression deregulation in human breast cancer . *Cancer Res* . 65 : 7065 - 7070 2005 ;
- 212 . Johnson SM , Grosshans H , Shingara J , Byrom M , Jarvis R , Cheng A , Labourier E , Reinert KL , Brown D , Slack FJ . RAS is regulated by the let-7 microRNA family . *Cell* . 120 : 635 - 647 2005 ;
- 213 . Akao Y , Nakagawa Y , Naoe T . let-7 microRNA functions as a potential growth suppressor in human colon cancer cells . *Biol Pharm Bull* . 29 : 903 - 906 2006 ;
- 214 . Yanaihara N , Caplen N , Bowman E , Seike M , Kumamoto K , Yi M , Stephens RM , Okamoto A , Yokota J , Tanaka T , Calin GA , Liu CG , Croce CM , Harris CC . Unique microRNA molecular profiles in lung cancer diagnosis and prognosis . *Cancer Cell* . 9 : 189 - 198 2006 ;
- 215 . Lee YS , Dutta A . The tumor suppressor microRNA let-7 represses the HMG2 oncogene . *Genes Dev* . 21 : 1025 - 1030 2007 ;
- 216 . Sampson VB , Rong NH , Han J , Yang Q , Aris V , Soteropoulos P , Petrelli NJ , Dunn SP , Krueger LJ . MicroRNA let-7a down-regulates MYC and reverts MYC-induced growth in Burkitt lymphoma cells . *Cancer Res* . 67 : 9762 - 9770 2007 ;
- 217 . Dong JT . Chromosomal deletions and tumor suppressor genes in prostate cancer . *Cancer Metastasis Rev* . 20 : 173 - 193 2001 ;
- 218 . Calin GA , Cimmino A , Fabbri M , Ferracin M , Wojcik SE , Shimizu M , Taccioli C , Zanesi N , Garzon R , Aqeilan RI , Alder H , Volinia S , Rassenti L , Liu X , Liu CG , Kipps TJ , Negrini M , Croce CM . MiR-15a and miR-16-1 cluster functions in human leukemia . *Proc Natl Acad Sci U S A* . 105 : 5166 - 5171 2008 ;
- 219 . Nasser MW , Datta J , Nuovo G , Kutay H , Motiwala T , Majumder S , Wang B , Suster S , Jacob ST , Ghoshal K . Down-regulation of micro-RNA-1 (miR-1) in lung cancer. Suppression of tumorigenic property of lung cancer cells and their sensitization to doxorubicin-induced apoptosis by miR-1 . *J Biol Chem* . 283 : 33394 - 33405 2008 ;
- 220 . Reddy SDN , Ohshiro K , Rayala SK , Kumar R . MicroRNA-7, a homeobox D10 target, inhibits p21-activated kinase 1 and regulates its functions . *Cancer Res* . 68 : 8195 - 8200 2008 ;
- 221 . Guessous F , Zhang Y , Kofman A , Catania A , Li Y , Schiff D , Purow B , Abounader R . microRNA-34a is tumor suppressive in brain tumors and glioma stem cells . *Cell Cycle* . 9 : 1031 - 6 2010 ;
- 222 . Yan RT , He L , Wang SZ . Pro-photoreceptor activity of chick neurogenin1 . *Invest Ophthalmol Vis Sci* . 50 : 5567 - 76 2009 ;
- 223 . Cole KA , Attiyeh EF , Mosse YP , Laquaglia MJ , Diskin SJ , Brodeur GM , Maris JM . A functional screen identifies miR-34a as a candidate neuroblastoma tumor suppressor gene . *Mol Cancer Res* . 6 : 735 - 42 2008 ;
- 224 . Jiang Q , Wang Y , Hao Y , Juan L , Teng M , Zhang X , Li M , Wang G , Liu Y . miR2Disease: a manually curated database for microRNA deregulation in human disease . *Nucleic Acids Res* . 37 : D98 - 104 2009 ;
- 225 . Igney FH , Krammer PH . Death and anti-death: tumour resistance to apoptosis . *Nat Rev Cancer* . 2 : 277 - 288 2002 ;
- 226 . Bush JA , Li G . The role of Bcl-2 family members in the progression of cutaneous melanoma . *Clin Exp Metastasis* . 20 : 531 - 539 2003 ;
- 227 . Coultas L , Strasser A . The role of the Bcl-2 protein family in cancer . *Semin Cancer Biol* . 13 : 115 - 123 2003 ;
- 228 . Reed JC , Jurgensmeier JM , Matsuyama S . Bcl-2 family proteins and mitochondria . *Biochim Biophys Acta* . 1366 : 127 - 137 1998 ;
- 229 . Henry-Mowatt J , Dive C , Martinou JC , James D . Role of mitochondrial membrane permeabilization in apoptosis and cancer . *Oncogene* . 23 : 2850 - 2860 2004 ;
- 230 . Li P , Nijhawan D , Budihardjo I , Srinivasula SM , Ahmad M , Alnemri ES , Wang X . Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade . *Cell* . 91 : 479 - 489 1997 ;
- 231 . Du C , Fang M , Li Y , Li L , Wang X . Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition . *Cell* . 102 : 33 - 42 2000 ;
- 232 . Verhagen AM , Ekert PG , Pakusch M , Silke J , Connolly LM , Reid GE , Moritz RL , Simpson RJ , Vaux DL . Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins . *Cell* . 102 : 43 - 53 2000 ;
- 233 . Chinnaiyan AM , O'Rourke K , Tewari M , Dixit VM . FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis . *Cell* . 81 : 505 - 512 1995 ;
- 234 . Eskes R , Desagher S , Antonsson B , Martinou JC . Bid induces the oligomerization and insertion of Bax into the outer mitochondrial membrane . *Mol Cell Biol* . 20 : 929 - 935 2000 ;
- 235 . Vecchione A , Croce CM . Apoptomirs: small molecules have gained the license to kill . *Endocr-Relat Cancer* . 17 : F37-50 - F37-50 2010 ;
- 236 . Cimmino A , Calin GA , Fabbri M , Iorio MV , Ferracin M , Shimizu M , Wojcik SE , Aqeilan RI , Zupo S , Dono M , Rassenti L , Alder H , Volinia S , Liu CG , Kipps TJ , Negrini M , Croce CM . miR-15 and miR-16 induce apoptosis by targeting BCL2 . *Proc Natl Acad Sci U S A* . 102 : 13944 - 13949 2005 ;
- 237 . Ventura A , Young AG , Winslow MM , Lintault L , Meissner A , Erkland SJ , Newman J , Bronson RT , Crowley D , Stone JR , Jaenisch R , Sharp PA , Jacks T . Targeted deletion reveals essential and overlapping functions of the miR-17 through 92 family of miRNA clusters . *Cell* . 132 : 875 - 886 2008 ;
- 238 . Ambs S , Prueitt RL , Yi M , Hudson RS , Howe TM , Petrocca F , Wallace TA , Liu CG , Volinia S , Calin GA , Yfantis HG , Stephens RM , Croce CM . Genomic profiling of microRNA and messenger RNA reveals deregulated microRNA expression in prostate cancer . *Cancer Res* . 68 : 6162 - 6170 2008 ;
- 239 . Petrocca F , Visone R , Onelli MR , Shah MH , Nicoloso MS , de Martino I , Iliopoulos D , Pillozzi E , Liu CG , Negrini M , Cavazzini L , Volinia S , Alder H , Ruco LP , Baldassarre G , Croce CM , Vecchione A . E2F1-regulated microRNAs impair TGFbeta-dependent cell-cycle arrest and apoptosis in gastric cancer . *Cancer Cell* . 13 : 272 - 286 2008 ;
- 240 . Yamakuchi M , Ferlito M , Lowenstein CJ . miR-34a repression of SIRT1 regulates apoptosis . *Proc Natl Acad Sci U S A* . 105 : 13421 - 13426 2008 ;
- 241 . Zhang CZ , Zhang JX , Zhang AL , Shi ZD , Han L , Jia ZF , Yang WD , Wang GX , Jiang T , You YP , Pu PY , Cheng JQ , Kang CS . MiR-221 and miR-222 target PUMA to induce cell survival in glioblastoma . *Mol Cancer* . 9 : 229 - 229 2010 ;
- 242 . le Sage C , Nagel R , Egan DA , Schrier M , Mesman E , Mangiola A , Anile C , Maira G , Mercatelli N , Ciafrè SA , Farace MG , Agami R . Regulation of the p27(Kip1) tumor suppressor by miR-221 and miR-222 promotes cancer cell proliferation . *EMBO J* . 26 : 3699 - 3708 2007 ;
- 243 . Xu C , Lu Y , Pan Z , Chu W , Luo X , Lin H , Xiao J , Shan H , Wang Z , Yang B . The muscle-specific microRNAs miR-1 and miR-133 produce opposing effects on apoptosis by targeting HSP60, HSP70 and caspase-9 in cardiomyocytes . *J Cell Sci* . 120 : 3045 - 3052 2007 ;
- 244 . Chan JA , Krichevsky AM , Kosik KS . MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells . *Cancer Res* . 65 : 6029 - 6033 2005 ;
- 245 . Papagiannakopoulos T , Shapiro A , Kosik KS . MicroRNA-21 targets a network of key tumor-suppressive pathways in glioblastoma cells . *Cancer Res* . 68 : 8164 - 8172 2008 ;
- 246 . Bitomsky N , Wethkamp N , Marikkannu R , Klempnauer KH . siRNA-mediated knockdown of Pdc4 expression causes upregulation of p21(Waf1/Cip1) expression . *Oncogene* . 27 : 4820 - 4829 2008 ;

- 247 . Sayed D , He M , Hong C , Gao S , Rane S , Yang Z , Abdellatif M . MicroRNA-21 is a downstream effector of AKT that mediates its antiapoptotic effects via suppression of Fas ligand . *J Biol Chem* . 285 : 20281 - 20290 2010 ;
- 248 . Ovcharenko D , Kelnar K , Johnson C , Leng N , Brown D . Genome-scale microRNA and small interfering RNA screens identify small RNA modulators of TRAIL-induced apoptosis pathway . *Cancer Res* . 67 : 10782 - 10788 2007 ;
- 249 . Fischer C , Schneider M , Carmeliet P . Principles and therapeutic implications of angiogenesis, vasculogenesis and arteriogenesis . *Handb Exp Pharmacol* . 157 - 212 2006 ;
- 250 . Sullivan DC , Bicknell R . New molecular pathways in angiogenesis . *Br J Cancer* . 89 : 228 - 31 2003 ;
- 251 . Kuehbacher A , Urbich C , Zeiher AM , Dimmeler S . Role of Dicer and Drosha for endothelial microRNA expression and angiogenesis . *Circ Res* . 101 : 59 - 68 2007 ;
- 252 . Suárez Y , Fernández-Hernando C , Pober JS , Sessa WC . Dicer dependent microRNAs regulate gene expression and functions in human endothelial cells . *Circ Res* . 100 : 1164 - 1173 2007 ;
- 253 . Wang S , Olson EN . AngiomiRs--key regulators of angiogenesis . *Curr Opin Genet Dev* . 19 : 205 - 211 2009 ;
- 254 . Wang S , Aurora AB , Johnson BA , Qi X , McAnally J , Hill JA , Richardson JA , Bassel-Duby R , Olson EN . The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis . *Dev Cell* . 15 : 261 - 271 2008 ;
- 255 . Fish JE , Santoro MM , Morton SU , Yu S , Yeh RF , Wythe JD , Ivey KN , Bruneau BG , Stainier DY , Srivastava D . miR-126 regulates angiogenic signaling and vascular integrity . *Dev Cell* . 15 : 272 - 84 2008 ;
- 256 . Nikolic I , Plate KH , Schmidt MH . EGFL7 meets miRNA-126: an angiogenesis alliance . *J Angiogenesis Res* . 2 : 9 - 2010 ;
- 257 . Lee DY , Deng Z , Wang CH , Yang BB . MicroRNA-378 promotes cell survival, tumor growth, and angiogenesis by targeting SuFu and Fus-1 expression . *Proc Natl Acad Sci U S A* . 104 : 20350 - 20355 2007 ;
- 258 . Würdinger T , Tannous BA , Saydam O , Skog J , Grau S , Soutschek J , Weissleder R , Breakefield XO , Krichevsky AM . miR-296 regulates growth factor receptor overexpression in angiogenic endothelial cells . *Cancer Cell* . 14 : 382 - 393 2008 ;
- 259 . Dews M , Homayouni A , Yu D , Murphy D , Seignani C , Wentzel E , Furth EE , Lee WM , Enders GH , Mendell JT , Thomas-Tikhonenko A . Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster . *Nat Genet* . 38 : 1060 - 1065 2006 ;
- 260 . Fasano P , D'Alessandra Y , Di Stefano V , Melchionna R , Romani S , Pompilio G , Capogrossi MC , Martelli F . MicroRNA-210 modulates endothelial cell response to hypoxia and inhibits the receptor tyrosine kinase ligand Ephrin-A3 . *J Biol Chem* . 283 : 15878 - 15883 2008 ;
- 261 . Chen Y , Gorski DH . Regulation of angiogenesis through a microRNA (miR-130a) that down-regulates antiangiogenic homeobox genes GAX and HOXA5 . *Blood* . 111 : 1217 - 1226 2008 ;
- 262 . Polisenio L , Tuccoli A , Mariani L , Evangelista M , Citti L , Woods K , Mercatanti A , Hammond S , Rainaldi G . MicroRNAs modulate the angiogenic properties of HUVECs . *Blood* . 108 : 3068 - 3071 2006 ;
- 263 . Hua Z , Lv Q , Ye W , Wong CKA , Cai G , Gu D , Ji Y , Zhao C , Wang J , Yang BB , Zhang Y . MiRNA-directed regulation of VEGF and other angiogenic factors under hypoxia . *PLoS One* . 1 : e116 - e116 2006 ;
- 264 . Valadi H , Ekstrom K , Bossios A , Sjostrand M , Lee JJ , Lotvall JO . Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells . *Nat Cell Biol* . 9 : 654 - 9 2007 ;
- 265 . Kosaka N , Iguchi H , Ochiya T . Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis . *Cancer Sci* . 101 : 2087 - 2092 2010 ;
- 266 . Thery C , Duban L , Segura E , Veron P , Lantz O , Amigorena S . Indirect activation of naive CD4+ T cells by dendritic cell-derived exosomes . *Nat Immunol* . 3 : 1156 - 62 2002 ;
- 267 . Keller S , Sanderson MP , Stoeck A , Altevogt P . Exosomes: from biogenesis and secretion to biological function . *Immunol Lett* . 107 : 102 - 108 2006 ;
- 268 . Skog J , Würdinger T , van Rijn S , Meijer DH , Gainche L , Sena-Estevés M , Curry WT Jr , Carter BS , Krichevsky AM , Breakefield XO . Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers . *Nat Cell Biol* . 10 : 1470 - 6 2008 ;
- 269 . Hunter MP , Ismail N , Zhang X , Aguda BD , Lee EJ , Yu L , Xiao T , Schafer J , Lee ML , Schmittgen TD , Nana-Sinkam SP , Jarjoura D , Marsh CB . Detection of microRNA expression in human peripheral blood microvesicles . *PLoS One* . 3 : e3694 - 2008 ;
- 270 . Caby MP , Lankar D , Vincendeau-Scherrer C , Raposo G , Bonnerot C . Exosomal-like vesicles are present in human blood plasma . *Int Immunol* . 17 : 879 - 887 2005 ;
- 271 . Rabinowitz G , Gercel-Taylor C , Day JM , Taylor DD , Kloecker GH . Exosomal microRNA: a diagnostic marker for lung cancer . *Clin Lung Cancer* . 10 : 42 - 6 2009 ;
- 272 . Taylor DD , Gercel-Taylor C , Parker LP . Patient-derived tumor-reactive antibodies as diagnostic markers for ovarian cancer . *Gynecol Oncol* . 115 : 112 - 20 2009 ;
- 273 . Pisitkun T , Shen RF , Knepper MA . Identification and proteomic profiling of exosomes in human urine . *Proc Natl Acad Sci U S A* . 101 : 13368 - 73 2004 ;
- 274 . Gonzales PA , Pisitkun T , Hoffert JD , Tchapyjnikov D , Star RA , Kleta R , Wang NS , Knepper MA . Large-scale proteomics and phosphoproteomics of urinary exosomes . *J Am Soc Nephrol* . 20 : 363 - 379 2009 ;
- 275 . Michael A , Bajracharya SD , Yuen PS , Zhou H , Star RA , Illei GG , Alevizos I . Exosomes from human saliva as a source of microRNA biomarkers . *Oral Dis* . 16 : 34 - 8 2010 ;
- 276 . Palanisamy V , Sharma S , Deshpande A , Zhou H , Gimzewski J , Wong DT . Nanostructural and transcriptomic analyses of human saliva derived exosomes . *PLoS One* . 5 : e8577 - e8577 2010 ;
- 277 . Keller S , Rupp C , Stoeck A , Runz S , Fogel M , Lugert S , Hager HD , Abdel-Bakky MS , Gutwein P , Altevogt P . CD24 is a marker of exosomes secreted into urine and amniotic fluid . *Kidney Int* . 72 : 1095 - 102 2007 ;
- 278 . Ohshima K , Inoue K , Fujiwara A , Hatakeyama K , Kanto K , Watanabe Y , Muramatsu K , Fukuda Y , Ogura S , Yamaguchi K , Mochizuki T . Let-7 microRNA family is selectively secreted into the extracellular environment via exosomes in a metastatic gastric cancer cell line . *PLoS One* . 5 : e13247 - 2010 ;
- 279 . Chen TS , Lai RC , Lee MM , Choo AB , Lee CN , Lim SK . Mesenchymal stem cell secretes microparticles enriched in pre-microRNAs . *Nucleic Acids Res* . 38 : 215 - 24 2010 ;
- 280 . Taylor DD , Gercel-Taylor C . MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer . *Gynecol Oncol* . 110 : 13 - 21 2008 ;
- 281 . Lehmann BD , Paine MS , Brooks AM , McCubrey JA , Renegar RH , Wang R , Terrian DM . Senescence-associated exosome release from human prostate cancer cells . *Cancer Res* . 68 : 7864 - 71 2008 ;
- 282 . Katakowski M , Buller B , Wang X , Rogers T , Chopp M . Functional microRNA is transferred between glioma cells . *Cancer Res* . 70 : 8259 - 63 2010 ;
- 283 . Collino F , Deregibus MC , Bruno S , Sterpone L , Aghemo G , Viltono L , Tetta C , Camussi G . Microvesicles derived from adult human bone marrow and tissue specific mesenchymal stem cells shuttle selected pattern of miRNAs . *PLoS One* . 5 : e11803 - 2010 ;
- 284 . Grange C , Tapparo M , Collino F , Vitillo L , Damasco C , Deregibus MC , Tetta C , Bussolati B , Camussi G . Microvesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niche . *Cancer Res* . 71 : 5346 - 56 2011 ;
- 285 . Purow B . The elephant in the room: do microRNA-based therapies have a realistic chance of succeeding for brain tumors such as glioblastoma? . *J Neurooncol* . 103 : 429 - 36 2011 ;
- 286 . Lammers P , Bader AG . The Therapeutic Potential of microRNAs . *Innovations in Pharmaceutical Technology* . 52 - 55 2011 ;
- 287 . Bader AG , Brown D , Stoudemire J , Lammers P . Developing therapeutic microRNAs for cancer . *Gene Ther* . 2011 ;
- 288 . Trang P , Medina PP , Wiggins JF , Ruffino L , Kelnar K , Omotola M , Homer R , Brown D , Bader AG , Weidhaas JB , Slack FJ . Regression of murine lung tumors by the let-7 microRNA . *Oncogene* . 29 : 1580 - 1587 2010 ;
- 289 . Ji Q , Hao X , Meng Y , Zhang M , Desano J , Fan D , Xu L . Restoration of tumor suppressor miR-34 inhibits human p53-mutant gastric cancer tumorspheres . *BMC Cancer* . 8 : 266 - 266 2008 ;
- 290 . Esau CC . Inhibition of microRNA with antisense oligonucleotides . *Methods (San Diego, Calif)* . 44 : 55 - 60 2008 ;
- 291 . Lanford RE , Hildebrandt-Eriksen ES , Petri A , Persson R , Lindow M , Munk ME , Kauppinen S , Orum H . Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection . *Science* . 327 : 198 - 201 2010 ;

- 292 . Medina PP , Nolde M , Slack FJ . OncomiR addiction in an in vivo model of microRNA-21-induced pre-B-cell lymphoma . Nature . 467 : 86 - 90 2010 ;
- 293 . Jiang P , Rao EY , Meng N , Zhao Y , Wang JJ . MicroRNA-17-92 significantly enhances radioresistance in human mantle cell lymphoma cells . Radiat Oncol . 5 : 100 - 2010 ;
- 294 . Chun-Zhi Z , Lei H , An-Ling Z , Yan-Chao F , Xiao Y , Guang-Xiu W , Zhi-Fan J , Pei-Yu P , Qing-Yu Z , Chun-Sheng K . MicroRNA-221 and microRNA-222 regulate gastric carcinoma cell proliferation and radioresistance by targeting PTEN . BMC Cancer . 10 : 367 - 2010 ;
- 295 . Allen KE , Weiss GJ . Resistance may not be futile: microRNA biomarkers for chemoresistance and potential therapeutics . Mol Cancer Ther . 9 : 3126 - 36 2010 ;
- 296 . Ranade AR , Cherba D , Sridhar S , Richardson P , Webb C , Paripati A , Bowles B , Weiss GJ . MicroRNA 92a-2\*: a biomarker predictive for chemoresistance and prognostic for survival in patients with small cell lung cancer . J Thorac Oncol . 5 : 1273 - 8 2010 ;
- 297 . Song B , Wang Y , Xi Y , Kudo K , Bruheim S , Botchkina GI , Gavin E , Wan Y , Formentini A , Kornmann M , Fodstad O , Ju J . Mechanism of chemoresistance mediated by miR-140 in human osteosarcoma and colon cancer cells . Oncogene . 28 : 4065 - 74 2009 ;
- 298 . Song B , Wang Y , Titmus MA , Botchkina G , Formentini A , Kornmann M , Ju J . Molecular mechanism of chemoresistance by miR-215 in osteosarcoma and colon cancer cells . Mol Cancer . 9 : 96 - 2010 ;
- 299 . Sorrentino A , Liu CG , Addario A , Peschle C , Scambia G , Ferlini C . Role of microRNAs in drug-resistant ovarian cancer cells . Gynecol Oncol . 111 : 478 - 86 2008 ;

**Figure 1**

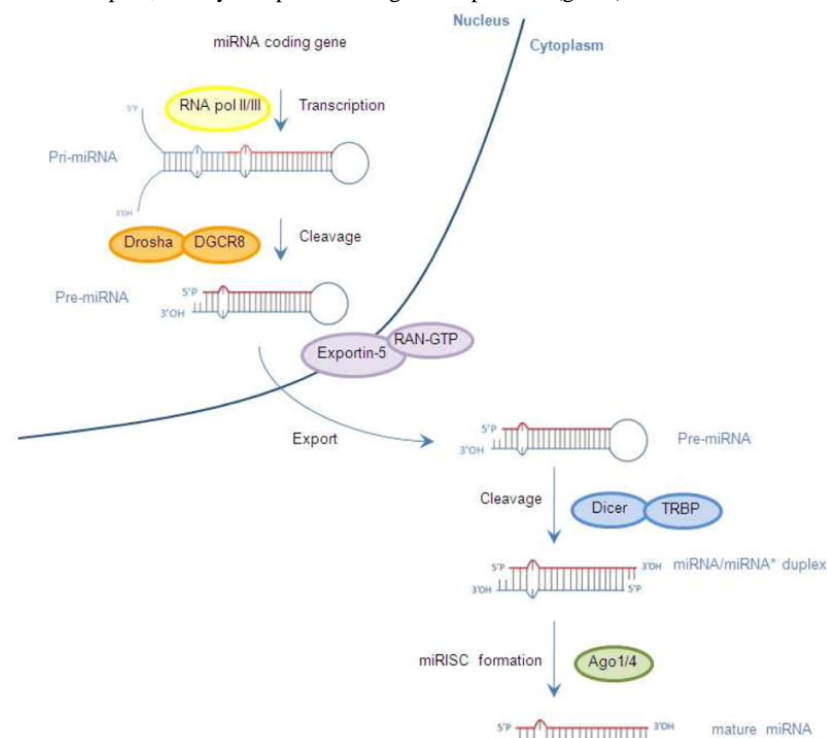
Time-dependent expression of lin-4 and let-7 microRNAs during *C. elegans* larval development and their impact on the expression of key proteins involved in larvae stage transitions.



**Figure 2**

The biogenesis mechanism of microRNAs

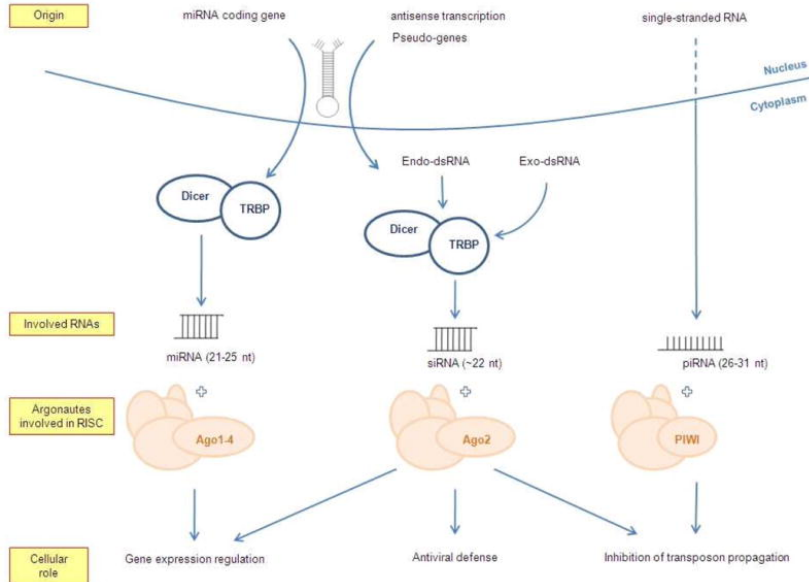
The transcription of a miRNA coding gene by a polymerase (yellow symbol) or the splicing of an intron generates a primary transcript called pri-miRNA. The cleavage of this transcript by the RNase Drosha associated with DGCR8 (orange) generates an RNA precursor called pre-miRNA. This precursor is then exported to the cytoplasm *via* the exportin-5 (purple) where it will be excised by the Drosha enzyme associated with the TRBP protein (blue). Only one of the two duplex strands of the miRNA, called the mature strand, is incorporated into the RISC complex, mainly composed of Argonaute proteins (green).



### Figure 3

#### Comparison of miRNAs, siRNAs, and piRNAs

miRNAs are processed according to the pathway described in Figure 2. siRNAs come from exogenous double-stranded RNA (Exo-dsRNA; viral infection) or endogenous double-stranded RNA (Endo-dsRNA; reverse transcription or transposons). Like miRNAs, siRNAs are matured by Dicer associated with TRBP and then incorporated into the RISC complex mainly composed of Ago2. siRNAs play a role in cell defense after a viral infection, in the inhibition of transposon propagation, or in the simple regulation of gene expression. piRNAs are different from miRNAs and siRNAs mainly because of their origin and their maturation; they are derived from single-stranded RNAs and are not processed by Dicer. They are also a few nucleotides longer than miRNAs and siRNAs, are mainly present in germinal cell lines, and contribute to maintaining the genome integrity targeting transposable elements.



### Figure 4

#### Localization of miRNA coding genes located in gene units coding for pre-mRNAs (31)

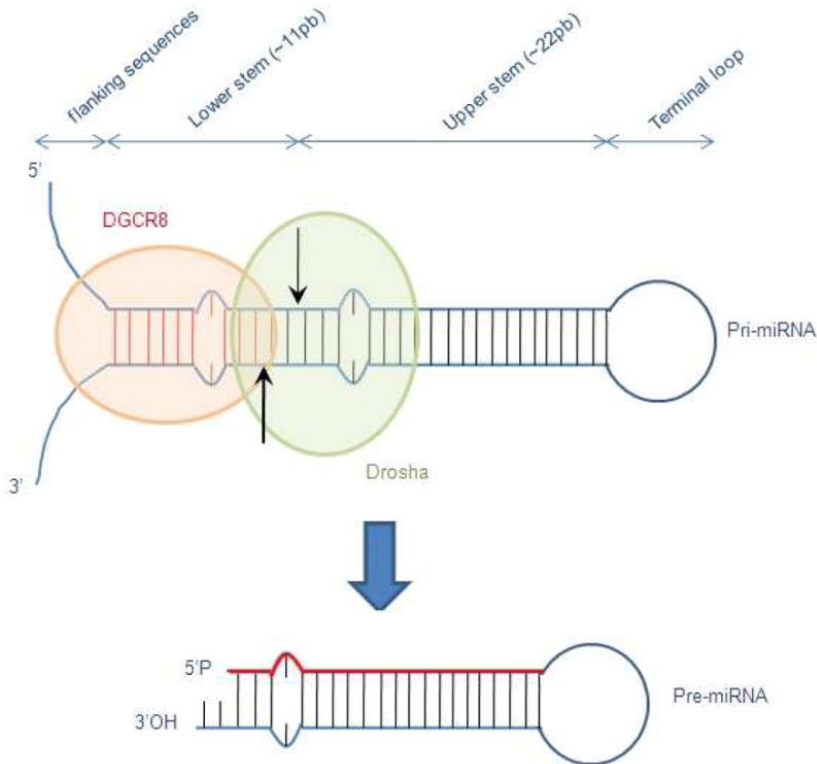
miRNAs can be located in transcription units not coding for proteins (A) or coding for proteins (B). miRNAs can also be located in either the intron (1) or exon (2).



**Figure 5**

Maturation of pri-miRNA into pre-miRNA by the Drosha/DGCR8 complex (43)

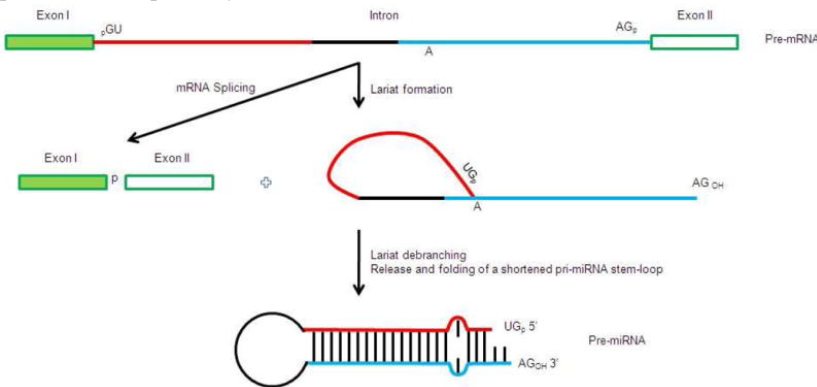
DGCR8 binds to double-stranded RNA, recognizes the pri-miRNA flanking sequences, and allows the stem-loop cleavage by the ribonuclease Drosha, approximately 11 bp from the junction between the flanking sequences and the stem-loop. This asymmetric cleavage leaves two unpaired nucleotides at the 3'OH end.



**Figure 6**

mirtron pathway (44,45)

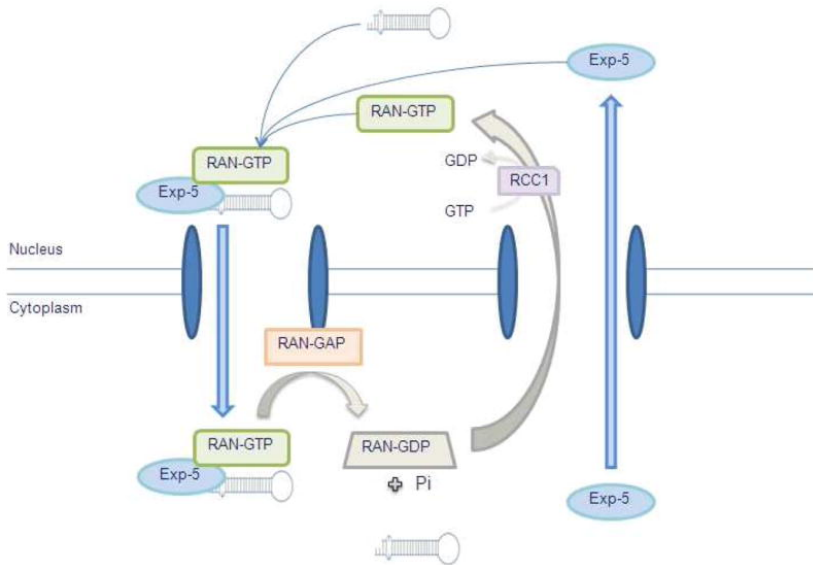
In the nucleus, short introns with hairpin potential, termed mirtrons, can be spliced and debranched into pre-miRNA hairpins. Hairpin potential is due to the complementarity of the sequences of the 5' and 3' domains of the introns (red and blue domains of the intron in the figure, respectively). The intron donor splicing site (GU) and the acceptor splicing site (AG) constitute the 5' end and the 3' end of the pre-mirtron, respectively.



**Figure 7**

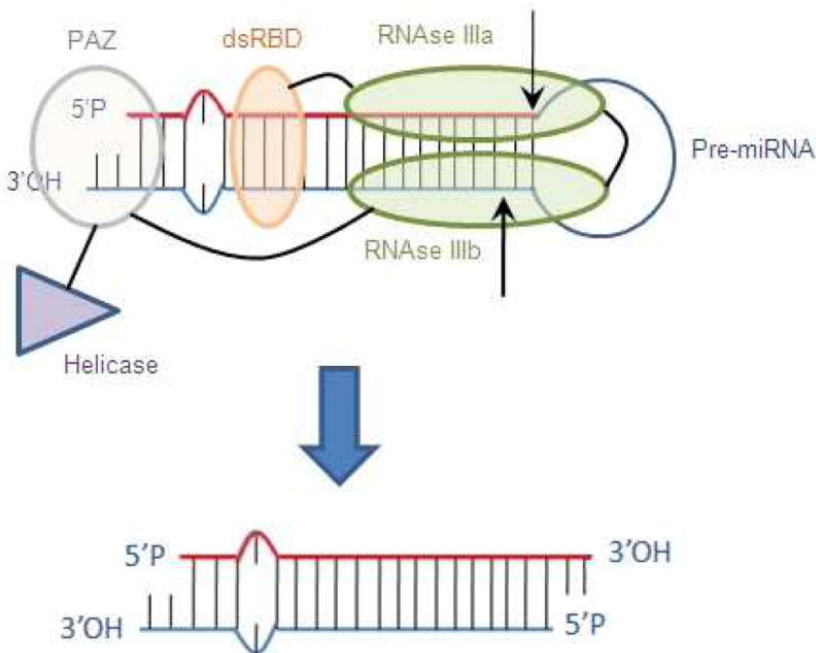
Mechanism of pre-miRNA export from the nucleus to the cytoplasm (48 )

The Exp-5/RAN-GTP/pre-miRNA complex binds to nucleoporins in the nucleus and goes through the nuclear pores. The release of pre-miRNAs into the cytoplasm is linked to the hydrolysis of RAN-bound GTP by the RAN-GTPase-activating protein. Exp-5 is then transported back into the nucleus and RAN-GTP is regenerated with RCC1 (Ran guanine nucleotide exchange factor).

**Figure 8**

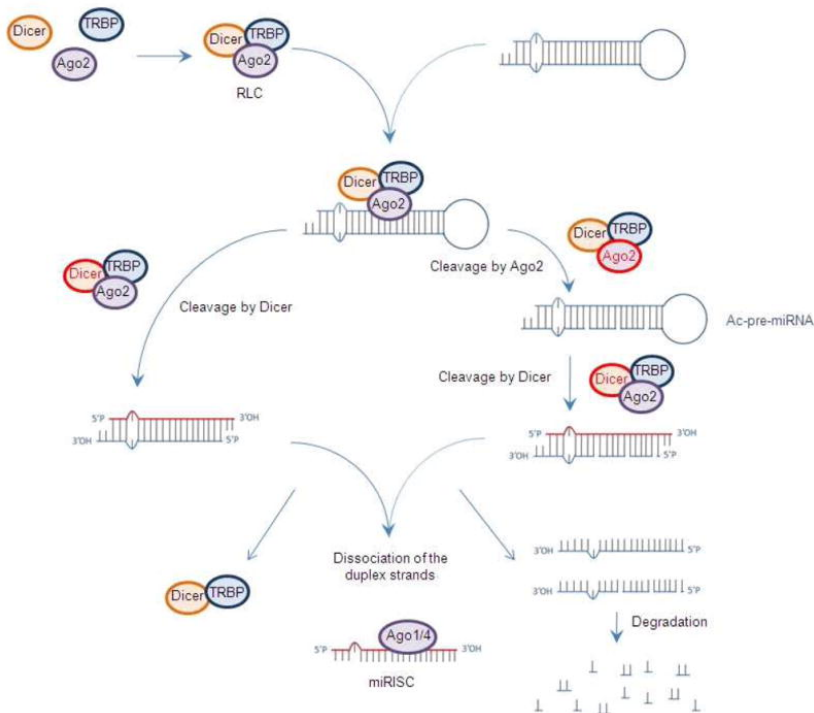
Dicer-dependent cleavage of pre-miRNA (59 )

The Dicer PAZ domain recognizes the single-stranded 2 nucleotide-long 3' overhang of the pre-miRNA. The dsRNA binding domain (dsRBD) allows the positioning of the RNase III domains and the cleavage of the pre-miRNA.



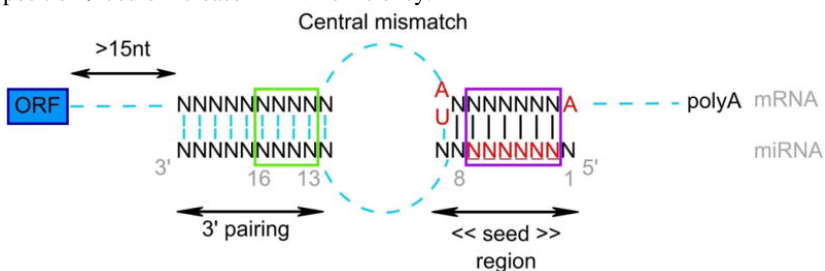
**Figure 9**  
miRISC formation (77 )

The RLC is composed of Dicer, TRBP, and Ago2. Two pathways may lead to the formation of miRISC. One (left) includes dissociation of the two strands of the duplex after cleavage of the pre-miRNA by Dicer. The second (right) first relies on the cleavage of the complementary strand by Ago2, allowing the formation of a transitory miRNA called Ac-pre-miRNA (Ago2-clived pre-miRNA) before cleavage by Dicer. In this model, the passenger strand is then degraded.



**Figure 10**  
Schematic model of the interaction between miRNAs and target mRNAs in metazoans (94 )

The major structural features are the following. miRNAs target mRNAs in the 3'UTR at a distance from the stop codon greater than 15 nt. A perfect complementarity is found in the seed region between nucleotides 2 and 7 from the 5' end of the miRNA (pink rectangle). A central bulge prevents endonucleolytic cleavage mediated by Ago2 (a major difference with the miRNA–target mRNA interaction in plants). A few nucleotide matches in the miRNA 3' end (especially between nucleotides 13 and 16) is necessary for the best stabilization of the miRNA/mRNA target duplex (green rectangle). The presence in the mRNA sequence of an A residue in position 1 and/or an A or U residue in position 9 could increase miRNA efficiency.





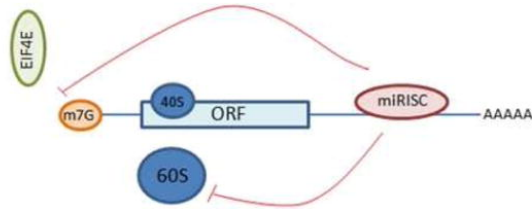


## Figure 12

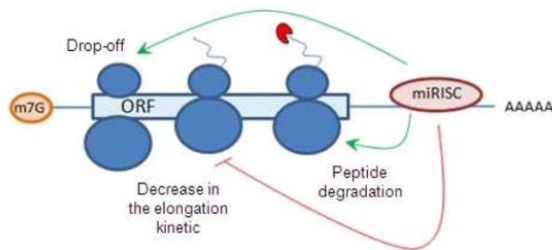
### Mechanisms of regulation of mRNA target translation by miRNA

(A) Regulation by translation initiation repression. This regulation can occur either by inhibition of eIF4E fixation at the cap because of a competition with Ago proteins or by inhibition of the recruitment of the 60S ribosomal subunit. (B) Regulation by repression at the translation postinitiation step. Three mechanisms have been described: regulation by premature release of ribosomes (drop-off), by decrease in the elongation kinetics, or by degradation of the newly synthesized peptide. (C) Regulation by mRNA degradation. A complex containing two deadenylases (CCR4 and CAF1) and NOT protein degrades the polyA tail. A decapping complex removes the cap at the 5' end and mRNA degradation can take place from 5' to 3' with the endonuclease Xrn1 or from 3' to 5' with exosomes.

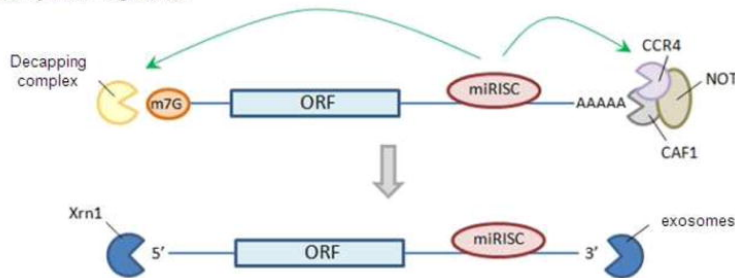
A. Initiation repression



B. Postinitiation repression



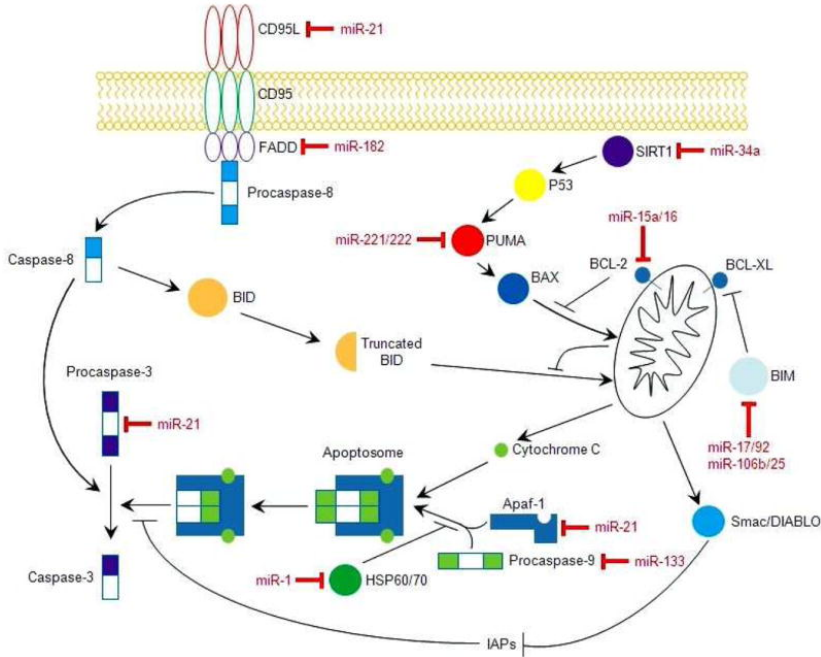
C. Regulation by mRNA degradation



### Figure 13

#### Implication of miRNAs in the apoptosis process (235 )

In the intrinsic pathway, the pro-apoptotic protein Puma is the target of miR-221/222 and is regulated by p53, target of the protein SIRT1, itself regulated by miR-34a. Anti-apoptotic protein Bcl-2 is the direct target of miR-15a/16, and Bcl-xL is the indirect target of some members of the miR-17/92 and miR-106b/25 clusters that regulate the pro-apoptotic protein Bim. Several miRNAs repress the apoptosome proteins, such as miR-21 targeting APAF-1 and miR-133 targeting caspase-9. miR-1 is a pro-apoptotic miRNA targeting HSP60 and HSP70, proteins that inhibit apoptosome formation. In the extrinsic pathway, concerning the CD95 receptor and its ligand CD95L (or FAS-L), miR-21 acts as a repressor of this ligand, whereas miR-182 lowers FADD expression. miR-21 also plays a role in caspase-3 regulation.



**Table 1**

Biocomputational tools for prediction of mRNA target sites in metazoans.

Biocomputational tools	Criteria	Websites	References
Targetscan	a ; d ; e ; f ; g	<a href="http://www.targetscan.org">http://www.targetscan.org</a>	(88 , 98 )
TargetscanS	a ; b (6-nt pairing necessary); d ; f ; g	<a href="http://www.targetscan.org">http://www.targetscan.org</a>	(91 )
PicTar	a ; c ; d ; e	<a href="http://www.pictar.mdc-berlin.de">http://www.pictar.mdc-berlin.de</a>	(99 )
MiRanda	a ; b ; d ; e	<a href="http://www.microna.org">http://www.microna.org</a>	(100 , 101 )
Microcosm targets	Based on MiRanda software	<a href="http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/">http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/</a>	(14 )
PITA Top	a ; b ; d ; e ; g ; h	<a href="http://genie.weizmann.ac.il/pubs/mir07/mir07_prediction.html">http://genie.weizmann.ac.il/pubs/mir07/mir07_prediction.html</a>	(102 )
EIMMo	a ; d ; e	<a href="http://www.mirz.unibas.ch/EIMMo2">http://www.mirz.unibas.ch/EIMMo2</a>	(103 )
Diana-microT	a ; b ; c ; d ; f	<a href="http://diana.cslab.ece.ntua.gr/microT">http://diana.cslab.ece.ntua.gr/microT</a>	(104 , 105 )

**a** Strong pairing in the seed region**b** Moderate pairing in the seed region**c** Thermodynamic stability**d** Interspecies preservation of target sites**e** Number of target sites on the 3'UTR of a given mRNA**f** Sequence context (location of the miRNA sites with regard to the stop codon and to the polyA tail or presence of AU-rich clusters)**g** Accessibility of the target site to the RISC complex**h** Possibility of tuning the search parameters