Abstract

MicroRNAs (miRNAs) are short, 20-22 nucleotide RNA molecules that function as negative regulators of gene expression in eukaryotic organisms. RNA mediated gene silencing pathways have essential roles in development, cell differentiation, proliferation, and cell death. It is becoming clear that microRNAs can play a very important role in regulation of gene expression. Understanding the basic mechanism of miRNA biogenesis is one of the central aims of molecular biologists in the future. MicroRNA molecules are produced from hairpin precursors formed from large transcripts. Two RNase III endonucleases, Drosha and Dicer, cleave both strands of the RNA and generate 20-22 nucleotide miRNA duplex. One arm of the duplex is selected and incorporated into RNA-induced silencing complex (RISC). RISC was identified earlier to repress the translation of mRNA in the presence of small interfering RNAs (siRNAs) by RNA interference (RNAi) mechanism. In animal cells, single-stranded miRNAs associated with RISC usually bind to target mRNAs through partial base-pairing, and result in translational repression. In plants, most miRNAs trigger mRNA cleavage through perfect base-pairing between miRNAs and target mRNAs. MiRNAs play important roles in development and diseases of plants and animals. There might be a possibility to use these microRNA signatures for a specific cancer classification with potential predictive and therapeutic value. MicroRNAs are aberrantly expressed in all studied cancer tissues. They are often located in cancer-associated genomic regions, act as tumor suppressors or oncogenes. For instance, miR-17–92, miR-155, miR-21, which are over expressed in tumors, might be considered as oncogenes. MiRNAs, such as let-7, which expression is reduced in cancer cells, can act as tumor-suppressor.

The aim of this paper is to summarize the main findings from research on miRNA biogenesis, functionality and cancer relevance. A narrative literature review of relevant papers known to the authors was conducted.
**Introduction**

MicroRNAs (miRNAs) comprise a new class of regulatory genes that function as 20–25 nucleotide (nt) single-stranded RNAs to control the expression of protein-coding genes (Bartel, 2004). The first miRNA, lin-4, and its protein target, lin-14, were discovered in C. elegans in the Ambros and Ruvkun labs. It was found that lin-4 gene did not encode proteins, but encode a 21 nucleotide RNA that partially base-pairs with multiple sites in the 3’ UTR of lin-14, causing inhibition of lin-14 protein expression (Lee et al, 1993; Wightman et al, 1993). Because the lin-14 mRNA level and polyribosome association appeared to be unchanged by lin-4, they suggested that miRNAs repress protein expression at the translational level (Olsen et al, 1999; Wightman et al, 1993). Further studies in C. elegans identified lin-28 as another target of lin-4, and lin-28 is regulated by lin-4 through translational inhibition as well (Moss et al, 1997; Seggerson et al, 2002).

Almost all scientists did not pay any attention to the new class of small RNAs until let-7 was discovered in C. elegans seven years latter. Let-7 regulates late larval development by inhibiting lin-41 expression (Reinhart et al, 2000; Slack et al, 2000). The sequence and developmental expression pattern of let-7 were highly conserved in a wide range of animal species, including vertebrate, ascidian, hemichordate, mollusk, annelid, and arthropod (Pasquinelli et al, 2000), suggesting that let-7 plays important roles in biological processes in animals. This finding attracted lots of attention from scientists. MiRNA lin-4 and let-7 are recognized as the founding members of miRNAs.

Each miRNA is thought to regulate multiple genes, hundreds of miRNA genes are predicted and several hundreds have been cloned and sequenced from C. elegans, Drosophila, Arabidopsis, mice and humans. The large number of miRNAs and homologous sequences of many miRNAs among organisms suggests that these RNAs
might constitute an abundant component of the gene regulatory machinery with an ancient origin. In the past few years, a huge amount of papers related to miRNA have been published, and miRNA related research has become the hottest research fields in biology. In 2006, the Nobel Prize in Physiology or medicine was awarded to Andrew Z. Fire and Craig C. Mello for their discovery of RNA interference-gene silencing by double-stranded RNA.

**Biogenesis of miRNA**

Most miRNA genes are located far from protein coding genes. They might have their own promoters and are transcribed independently. However, some miRNA genes, 1/4 of human miRNA genes are located in introns (Lee et al., 2003). They are transcribed along with the protein coding genes, and are spliced out during processing. Because miRNA genes and their host protein genes are co-transcribed, their relationship is often conserved in evolution. MiRNAs do not show a lot variety among different species. Some of miRNA genes are clustered on chromosomes. These are transcribed from a shared promoter. Although this kind of transcription is seldom in nematodes and human beings (Lim et al., 2003a, 2003b), half of the miRNA genes in Drosophila are found clustered (Aravin et al., 2003). Those miRNAs genes which are clustered usually have relationships with each other. For example, the human mir-15a-mir-16 gene cluster is located in an anti-oncogene on chromosome 13, and down-regulation of these miRNAs caused leukemia (Calin et al, 2002). This indicates that both miR-15a and miR-16 are related to the cancer (Calin et al, 2002).

Currently, we still know little about how the miRNA genes are transcribed to pri-miRNAs\(^1\). It is proposed that both the RNA polymerase II and III can participate in miRNA transcription. There is no doubt that the miRNA genes located in the protein gene introns are transcribed by the polymerase II. Although most of other

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\(^1\) pri-miRNA: primary microRNA, several hundred nucleotides long miRNA precursor.
miRNA genes lack the signal of polyadenylation (Ohler et al., 2004), we still have some indirect proofs which indicate that they may also be the transcription products of polymerase II (Bartel, 2004):

A. Some pri-miRNAs are very long, sometimes longer than 1 kb. They are longer than the transcription products of polymerase III.

B. The internal runs of uridine residue in some pri-miRNAs would be expected to prematurely terminate the polymerase III transcription.

C. Many miRNA genes are expressed during the process of growth. This is more familiar in polymerase II than in polymerase III.

D. Fusions were made by placing the open reading frame of the reporter gene downstream from the 5’ portion promoter of miRNA genes. Abundant reporter gene expression was observed. It is suggested that the pri-miRNA transcripts are 5’ capped which is the characteristic of polymerase II transcripts (Johnson et al., 2003).

The first step of the pri-miRNA cleavage is completed by the RNase III Drosha which generates ~70 nucleotides pre-miRNA2 (figure 1). The pre-miRNA is formed as a stem loop structure and has 5’ phosphate and 3’ ~2 nucleotides overhang which is formed by the Drosha cleavage. Drosha contains 2 tandem RNase III domains: one dsRNA binding domain, another amino terminal segment of unknown function (Lee et al., 2003). The efficiency of Drosha processing is determined by its terminal loop size; stem structure, and flanking sequence of the Drosha cleavage site (Lee et al., 2003; Zeng, 2003). Drosha is an important component in the process of pri-miRNA cleavage, but there is also another protein Pasha. We still don’t clearly know the function of Pasha, but it may participate in identifying the transcripts of miRNA genes and incorporate them into the complex (Zamore et al, 2005).

After pre-miRNAs are formed in the nucleus, they are transported into cytoplasm by Exportin 5 (Exp 5) which is a Ran-GTP dependent nucleo/cytoplasmic cargo

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2 Pre-miRNA: precursor microRNA, an 70–nt intermediate with stem loop miRNA precursor.
transporter (figure 1). As a result of the high concentration of Ran-GTP in nucleus, pre-miRNAs are released from Drosha and integrated into Exportin 5. When pre-miRNAs are transported into the cytoplasm where the Ran-GTP concentration is lower; then pre-miRNAs are released from Exportin 5 (Bohnsack et al, 2004).

![Figure 1: The biogenesis of miRNA (Adapted form Bartel 2004).](image)

1. Pri-miRNA is transcribed from miRNA genes.
2. Pri-miRNA is cleaved by Drosha to pre-miRNA.
3. Pre-miRNA is transported into cytoplasm.
4. Pre-miRNA is cleaved by Dicer to miRNA: miRNA* duplex.
5. MiRNA: miRNA* duplex is unwound by Helicase.
6. MiRNA and miRNA* are selectively incorporated into the RISC.

Once the pre-miRNAs are transported into cytoplasm, the maturation of miRNA is processed by another RNase III enzyme Dicer (Lee et al., 2003) (figure 1). Dicer was first discovered in generating siRNAs\(^3\) in RNAi (Bernstein et al., 2001). Later it is found that it is also the key enzyme in the maturation of miRNAs (Grishok et al., 2001; Hutvagner et al., 2001; Ketting et al., 2001). The process Dicer performs in

\(^3\) siRNAs: short interfering RNAs, exogenous, 21-23 bp, dsRNA.
miRNA maturation is similar to the formation of dsRNA in RNA interference. Dicer contains 2 tandem RNase III domain, a putative helicase domain, a DUF283 domain, and a PAZ (Piwi-Argonaute-Zwille) domain (Bernstein et al, 2001). The PAZ domain recognizes the end of the Drosha cleavage, and therefore positions the site of the second RNase III cleavage on the stem structure of the miRNA precursors. In the process of cleavage, firstly, Dicer recognizes the double strand portion of pre-miRNAs, probably with special affinity to the 5’ phosphate and 3’ 2 nucleotides overhang generated by Drosha (Bartel 2004). Then it cuts both strands of the duplex, leaving 5’phosphate 3’ 2 nucleotides overhang, which is similar to siRNA but with imperfect complementarity. This new formed duplex is called miRNA:miRNA* duplex.

**RISC assembly**

MicroRNAs were first reported to reside in miRNP\(^4\) which is short for microRNA ribonucleoprotein complex. In human, miRNP consists of eIF2C2, Gemin3, and Gemin4 (Mourelatos, et al, 2002). EIF2C2 was later found to be a constituent of the human siRNA- programmed RISC (Martinez, et al, 2002). Furthermore, the human let-7 miRNA is found to be associated with eIF2C2, and is able to guide cleavage of an artificial target with perfect complementarity to the miRNA (Hutvagner, et al, 2002). Thus, the miRNP is referred to as the RISC\(^5\). It is also supported by the evidence that plant miRNAs can direct cleavage of their targets (Llave, et al, 2002b; Tang, et al, 2003), and siRNA can also mediate translational repression (Doench, et al, 2003; Zeng, et al, 2003).

MiRNAs and siRNAs present in functional RISC have to be single stranded for pairing with the target RNA. There is a bias in the strand selection to incorporate into

\(^4\) miRNP: micro ribonucleoprotein complex.

\(^5\) RISC: RNA induced silencing complex.
the RISC. The strand incorporated into the RISC or selected as a miRNA is generally the one whose 5’ terminus is thermodynamically less stable in the duplex. Comparative sequence analysis demonstrated that the 5’ end of mature guiding strands have significantly lower thermodynamic stability compared to the 5’ end of the passenger strands in the stem of the pre-miRNA (Hutvagner, 2005). Transfection experiments in human cells show that chemically synthesized siRNAs which are efficient in gene silencing have similar thermodynamic profiles. The 5’ end of the guiding strands of the competent siRNAs is less stable than the 5’ end of the passenger strands (Khvorova, et al, 2003). In vitro experiments with chemically synthesized siRNAs and radio labeled RNA targets in Drosophila cell-free embryo extracts came to the same conclusion and provide additional insight into the mechanism of RISC assembly (Schwarz, et al, 2003). The relative thermodynamic strength of the first four nucleotide pairs of the 5’ end can be calculated by the nearest-neighbor method which monitors the thermodynamic properties of the two 5’ ends of siRNA. If a G:C base pair is altered to an I:C base pair in a closely symmetric siRNA, the modified strands will be more preferable for RISC assembly (Schwarz, et al, 2003).

Before the formation of the active regulatory complex, the RISC loading complex (RLC) places single stranded miRNA or siRNA into RISC (figure 2). In Drosophila, it is found that in RLC the Dcr-2-R2D2 heterodimer senses the differential free energy of the duplex ends, and determines which strand is selected to incorporate into the RISC (Tomari, et al, 2004). Dcr-2 is a member of the Dicer family. R2D2 (shown as DadR in figure 2) binds to the more thermodynamically stable strand (passenger strand), and its binding is facilitated by the characteristic 5’ phosphate. The cleavage of the passenger strand requires the Argonaute catalytic activity. Argonaute proteins

6 Guiding strand: also called anti-sense strand, the strand incorporated into the RISC, its 5’ end is less thermodynamically stable.
7 Passenger strand: also called sense strand, the strand is eliminated after the guiding strand incorporated into the RISC, its 5’ end is more thermodynamically stable.
8 Argonaute: members of a highly conserved family of proteins involved in RNAi and miRNA pathways.
are the core proteins in every described RNAi\(^9\) complex. They are approximately 100kDa proteins sometimes called PPD proteins. They have two conserved domains, the PAZ domain, which was proposed to bind single stranded RNA (Lingel, et al, 2003; Song, et al, 2003; Yan, et al, 2003), and the PIWI motif, which shows structural homology to the active center of RNase H (Ma, et al, 2005; Parker, et al, 2004; Parker, et al, 2005; Song, et al, 2004). Evidence supports that Argonaute proteins physically cooperates with small RNA loading machinery. In humans, the dsRNA binding domain of Dicer directly interacts with the PIWI domain of Ago2 protein (Pillai, et al, 2004). Some other RISC associated proteins including VIG, Fragile X-related protein, and the nuclease Tudor-SN do not have defined roles in the RISC (Caudy, et al, 2002, 2003; Ishizuka, et al, 2002). They could be accessory factors that modify the specificity or function of the RISC (Bartel, 2004). The RISC endonuclease, known as Slicer, is recruited after the other proteins in RISC have interacted with small RNAs (Bartel, 2004).

After the guiding strand is selected to incorporate into the RISC, the passenger strand with higher 5’ end free energy profile is eliminated (figure 2). It is showed that the passenger strand is eliminated after unwinding in an in vitro experiment in fly embryo extract (Schwarz, et al, 2003), and it is through the same activity that Ago protein cleave the cognate RNA target (Lau, et al, 2005). It was doubted if the passenger strand was too small for Ago protein to target the mRNA. However, evidence shows that the exogenously added passenger strand can be cleaved by affinity purified human Ago2 containing single stranded RNA (Martinez, et al, 2004).

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\(^9\) RNAi: RNA interference, the phenomenon whereby a dsRNA trigger leads to a sequence homology-dependent gene silencing.

**Post-transcriptional regulation by miRNA**

Both miRNAs and siRNAs are able to target mRNA degradation or translation silencing in mammalian cells and plants (Hutvagner, et al, 2002b; Llave et al, 2002; Doench, et al, 2003; Zeng, et al, 2003) (figure 3). The choice is determined by the identity to the mRNA target (Bartel, 2004). If the mRNA target has perfect or nearly perfect complementarity to the miRNA, the miRNA will specify mRNA cleavage. Otherwise, miRNA will repress translation when mRNA target does not have

**MiRNA-directed mRNA degradation**

*MiRNAs and siRNAs can direct endonucleolytic cleavage of mRNAs.*

The way that miRNAs and siRNAs control post-transcriptional gene expression is by directing endonuclease cleavage of the target mRNA. Such endonuclease cleavage was first demonstrated in cell cultures with exogenously provided dsRNAs (Tuschl et al, 1999; Hammond et al, 2000). But now, it is appreciated that some endogenous miRNAs in both plants and metazoans direct endonucleolytic cleavage (Llave et al. 2002; Yekta et al, 2004). Although some mismatches can be tolerated and still allow cleavage to occur, endonucleolytic cleavage is generally favored by perfect base-pairing between the miRNA and the mRNA. What should be payed attention to is the fact that base-pairing between the miRNA and the mRNA is not always sufficient to induce cleavage. It suggests that there can be additional requirements for a RISC complex to catalyze endonucleolytic cleavage (Chen 2004). Additional for the
The RISC complex is that there exists a specific Argonaute protein present within the RISC. In mammalian cells, both miRNAs and siRNAs are thought to be loaded into the same RISC, where they guide mRNA degradation or translation silencing depending on the complementarity of the target. In Drosophila, Argonaute2 (AGO2), as part of the RISC complex, is essential for siRNA mediated RNAi in embryos (figure 4), and is required for the assembly of siRNA into RISC in embryos (Okamura et al. 2004). In contrast, Argonaute1 (AGO1), another Argonaute protein in fly, which is dispensable for siRNA-directed target RNA cleavage in RNAi (figure 4), is required for mature miRNA production that impacts on miRNA-directed RNA cleavage (Okamura et al. 2004) (figure 5). The association of AGO1 with Dicer-1 and pre-miRNA also suggests that AGO1 is involved in miRNA biogenesis (Okamura et al. 2004).

Figure 4: AGO2 is necessary for siRNA mediated RNAi (Okamura et al. 2004).

When AGO2 is suppressed by introducing specific dsRNA in S2 cells expressing EGFP, the ability of the cells to silence EGFP by RNAi is severely reduced. In contrast, when AGO1 is suppressed, the EGFP silencing effect is unaffected, indicating that AGO1 is not essential for the RNAi pathway in S2 cells.

The general cellular mRNA degradation machinery

The products of RNAi cleavage seems to be degraded by the same enzymes that degrade cellular mRNA. Eukaryotic cells contain two general and conserved pathways for the degradation of bulk mRNA, both of which require an initial removal of the 3’ poly (A) tail in a process referred to as deadenylation (Parker and Song 2004). Deadenylation is followed by 3’ to 5’ exonucleolytic degradation by the
AGO1 is necessary for miRNA mediated RNAi (Okamura et al. 2004).

In vitro RNAi assays with lysates prepared from 14- to 16-h yw, AGO2\textsuperscript{k08121}, and AGO1\textsuperscript{k08121} embryos.

SiRNA directed its cleavage in AGO1\textsuperscript{k08121} embryo lysate as effectively as in wild type. However, cleavage of the target RNA directed by miR-2b was suppressed in AGO1\textsuperscript{k08121} embryo lysate by a factor of five-fold compared with that in wild type, suggesting that AGO1 is necessary for efficient target RNA cleavage mediated by miRNAs.

Exosome, a multimeric complex with 3’ to 5’ exonuclease activity. After deadenylation, mRNAs can be decapped by the Dcp1/Dcp2 decapping enzymes and degraded 5’ to 3’ by the abundant 5’ to 3’ exoribonuclease. Following mRNA cleavage triggered by siRNAs or miRNAs, the 3’ fragment is degraded by major cellular 5’-to-3’ exonucleases. (Valencia-Sanchez et al. 2006).

The degradation mode of the 5’ fragment from miRNA-induced cleavage is not so clear, and this fragment may be subject to two alternative fates. The first is that the 5’ fragment can be a substrate for the exosome; the second fate of the 5’ product can be the addition of a 3’ tail after the site of cleavage that includes predominantly Us, but can include As and Cs (Shen et al., 2004). This uridinylation occurs in both plants and
animals and could be a mechanism to enhance degradation for poor substrates for the exosome, which might require a 3’ extension to activate the exosome (Valencia-Sanchez et al. 2006). Alternatively, uridinylation may compete with 3’ to 5’ degradation, and substrates where uridinylation occurs could end up being targeted for decapping and 5’ to 3’ degradation.

*MiRNAs can target mRNAs for slicer-independent decay*

MiRNAs can also target mRNAs for increased decay by a slicer-independent mechanism. An example in which miRNA can target mRNAs for decay is from the analysis of mRNA decay stimulated by an AU-rich 3’ UTR regulatory sequence (ARE). AREs are a class of sequences that control mRNA decay rates and translation in eukaryotic cells (Espel 2005). Recent studies suggested that the miR-16 functions with RISC and the sequence specific RNA binding protein to target an ARE containing mRNA for degradation (Jing et al, 2005). From the case of AU-rich 3’ UTR regulatory sequence (ARE)-mediated degradation, it suggests that the miRNA/RISC complex is not involved in exosome-mediated decay because RNAi inhibition of exosome function has no impact (Valencia-Sanchez et al. 2006). Evidence that miRNAs might target mRNAs for decapping has come from comparing the subcellular distribution of Argonaute proteins with the decapping machinery. For eukaryotic cells, including yeast and mammals, the decapping enzymes are concentrated in specific cytoplasmic foci known as cytoplasmic processing bodies, also called P-bodies (Box 1), can be sites of mRNA decapping and degradation (Cougot et al, 2004). One of the Argonaute family members in C.elegans ALG-1(AGO-1) can accumulate in P-bodies (Ding et al, 2005). The mRNA targets of miRNAs also accumulate within P-bodies in a miRNA-dependent way (Liu et al. 2005b). It was suggested that miRNAs target mRNAs into P-bodies, which increased their association with the decapping machinery and thereby potentially reduce their levels by decapping and 5’-to-3’ degradation (Valencia-Sanchez et al. 2006).

In summary, miRNA combined with Argonaute proteins, either target mRNAs to
promote mRNA decapping followed by 5’ to 3’ degradation, or to a clearly defined decay pathway.

MiRNA-directed translational repression

The mechanism of how miRNA induces the inhibition of translation is currently unknown. Efficient translation repression by miRNAs often requires multiple miRNA binding sites (Bartel, et al, 2004). The events during translation can be generally divided into three stages: initiation, elongation and termination. Several protein factors are involved at each stage. The available evidence indicates that miRNAs can repress translation at both initiation and post-initiation levels (figure 6). Some reports indicated that Argonaute proteins, miRNAs and mRNAs repressed by miRNAs all accumulate in discrete P-bodies (also known as GW bodies) (Sen, et al, 2005; Liu, et al, 2005; Meister, et al, 2005).

Initiation is generally a rate-limiting step in translation and is frequently a subject of elaborate regulation, either affecting a specific mRNA or one with a global character (Pillai, et al, 2007). Evidence indicates that miRNAs can affect translation initiation which is supported by the fact that alterations in the translation initiation process can make an mRNA resistant to miRNA induced translation repression. Tethering of the translation factors eIF-4E or eIF-4G to an mRNA makes it resistant to miRNA induced repression (Pillai, et al, 2005). Tethered translation initiation factors will still be repressed if miRNAs affect translation at a step after translation initiation. Some experimental results support that translation can be inhibited by miRNA at the initiation step (Pillai, et al, 2007). Polysome profile\(^{10}\) analysis of luciferase reporter mRNAs, expressed from transfected plasmid DNA and repressed by either endogenous let-7 miRNP or miRNA independent tethering of Ago proteins to a reporter mRNA, showed a marked shift of the repressed mRNAs towards the lighter fractions of a sucrose gradient, indicative of an effect at the initiation step of

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\(^{10}\) Polysome profile: Density gradient centrifugation of cytoplasmic extracts used to study the association of miRNAs with translational machinery.
translation (Pillai, et al, 2005). Another experiment was taken with an endogenous miRNA target, cationic amino acid transporter 1 (CAT-1), which is regulated by miR-122 in liver and hepatoma cells. Under stress condition, when the miRNA mediated repression is relieved, a fraction of CAT-1 RMA shift from the lighter part of the gradient to the heavy region (Bhattacharyya, et al, 2006). Some other experiments suggest that miRNA can affect initiation by involving the m\(^7\)G cap\(^{11}\) recognition (Pillai, et al, 2007). When the repression occurs at the initiation step of translation, run-off of ribosomes results in aggregation of the repressed ribosome-free mRNA into P-bodies for either degradation or storage. (Liu, et al, 2005) (figure 6a)

Box 1: P-bodies

P-bodies have been established as sites of translational repression and mRNA decay, and they are enriched in factors involved in these pathways (Anderson, et al, 2006; Coller et al, 2004). The decay factors include the deadenylase complex Ccr4:Not1, the decapping complex Dcp1:Dcp2, the 5’-3’ exonuclease Xrn1 (Parker, et al, 2004). The proteins in P-bodies with repressive activities on translation include Dhh1p, Rck/p54, elf4E-T and Part1p. Ribosomes and translation initiation factors do not exist in P-bodies, with the exception of elf4E. GW182, a protein with glycine-tryptophan repeats (GW repeats) is required for P-body integrity. Alternatively, GW182 could be involved in localizing miRNA targets to P-bodies or facilitating the RISC remodeling steps required for the silencing and/or decay of these targets (Jajymiy et al, 2005; Liu et al, 2005b; Meister, et al, 2005; Rehwinkel et al, 2005). There is an inverse correlation between polysome association and P-bodies accumulation of mRNAs (Bhattacharyya, et al, 2006; Pillai, et al, 2005; Coller, et al, 2005; Teixeiria, et al, 2005; Brengues, et al, 2005). A block in the initiation step of translation leads to an increase in P-bodies size and number, due to the accumulation of inhibited miRNAs (Teixeiria, et al, 2005). On the other hand, drugs such as actinomycin D, which blocks mRNA synthesis, or cycloheximide, which retains mRNA on Polysomes, result in a loss of P-bodies (Andrei, et al, 2005; Cougot, et al, 2004). The fate of mRNAs accumulated in P-bodies depends on following general reactivation of cellular protein synthesis or in response to environmental stimuli. MRNAs can exit P-bodies and re-enter translation (Brengues, et al, 2005). As a result, P-bodies can function as temporary storage sites for inhibited mRNAs.

\(^{11}\) M\(^7\)m\(^7\)G cap: 7-methylguanylate cap of he 5’ end of nascent RNA transcripts.
There could be two possible mechanisms of translational post-initiation repression by miRNAs. One is that ribosomes on the mRNA are slowed or stalled by miRNA (figure 6b), the other is that translation continues at the same rate but nascent polypeptide chains are specifically degraded by protease (figure 6c). The sucrose gradient and metrizamide-buoyant density gradients of the lin-4 target mRNAs remained largely unchanged under conditions of repression, suggesting that mRNAs are successfully loaded with ribosomes and that repression affects post-initiation steps of translation (Olsen, et al, 1999; Seggerson, et al, 2002). In this model, repressed mRNAs remain largely associated with ribosomes and thus might not relocate to P-bodies. Alternatively, association of repressed mRNAs with ribosomes could be caused by the ability of RISC to recruit proteolytic enzymes that would degrade nascent polypeptides from the actively translating ribosomes. However, the proteolysis might be fast, and occur early on short nascent peptides. Premature translation termination could yield incomplete, aberrant proteins undergoing rapid degradation. The inability to visualize nascent polypeptides might also indicate that they are simply not being synthesized, possibly owing to a block in translation initiation (Pillai, et al, 2007).
Figure 6: Possible mechanisms of miRNA-mediated repression of target mRNAs in metazoan cells. (Pillai, et al, 2007)


(b). Post-initiation mechanism representing repression of translation at the elongation or termination step, or promotion of ribosome drop off (Peterson et al, 2006). In this model, mRNAs remain largely associated with ribosomes and thus might not relocate to P-bodies.

(c). Post-initiation mechanism involving rapid proteolysis of nascent polypeptide chains. There is no experimental support for this model.

MiRNA VS siRNA

Fundamentally, miRNAs and siRNAs are similar in terms of their molecular characteristics, biogenesis and functions. They are generally 21-25 nucleotide small RNA molecules. Both of them are selectively incorporated into the silencing complex RISC, and bind to target mRNAs for post-transcriptional repression (figure 3). They share a common RNase-3 processing enzyme, Dicer, to form mature miRNAs or siRNAs. They can trigger a biological process, RNAi, which is sequence-specific gene silencing mechanism that is induced by dsRNA. They can also target mRNAs for translational repression.

However, miRNAs and siRNAs are different in many facts, particularly in regard to their origin, evolutionary conservation, and the types of genes that they silence. First, miRNA derive from genomic loci distinct from other recognized genes, whereas siRNAs originate from mRNAs, transposons, viruses, or heterochromatic DNA (Bartel, 2004). Second, miRNAs are processed from pre-miRNAs by Dicer, and pre-miRNAs which have stem hairpin structure derive from pri-miRNA by Drosha (figure 1), whereas siRNAs in C. Elegans and D. Melanogaster are processed from long double stranded RNA duplex or extended hairpins (Bartel, 2004). Third, one single miRNA:miRNA* duplex is generated from one arm of each pre-miRNA molecule, whereas multiple siRNA duplexes are generated from each siRNA precursor molecules (in lower organisms). Fourth, miRNA sequences are conserved in related organisms, whereas endogenous siRNA sequences are rarely conserved (Ambros, et al, 2003a). Fifth, in many cases, miRNAs bind to the target mRNA 3’ UTRs through imperfect complementarity at multiple sites, and therefore negatively regulate target expression at the translational level. By contrast, siRNA often from a perfect duplex with their targets at only one site, and therefore direct the cleavage of the target RNAs at the site of complementarity. However, there are some exceptions: miR-172, all characterized plant miRNAs anneal to their targets with nearly perfect complementarity at a single site, either in the coding region or in the UTRs, therefore
directing their target mRNAs to destruction by cleavage and degradation (Chen, 2004; Llave, et al, 2002; Rhoades, et al, 2002). A similar example is that miR-196, nearly perfect base pairing to its target Hoxb8, directs cleavage of Hoxb8 mRNA both in mouse embryos and in cell culture (Yekta, et al, 2004). Conversely, when siRNAs do not have sufficient complementarity to their targets, siRNAs can trigger translational repression rather than mRNA cleavage in mammalian tissue culture (Doench, et al, 2003). Sixth, another distinction can be made between miRNAs and siRNAs: miRNAs specify the silencing genes which are very different or far from the genes that miRNAs are produced from. By contrast, endogenous siRNAs typically specify the silencing of the same or very similar loci from which they originate (Bartel, 2004). For example, viruses, transposons, and the heterochromatic outer repeats of centromeres are silenced by the siRNAs which are generated from them.

Although the two classes of silencing RNAs are difficult to be distinguished by either chemical composition or mechanism of action, distinctions above can be made to classify the small RNAs. As the fifth point mentioned above, the mechanism of silencing is mainly determined by the degree of complementarity, not by the fact that the small silencing RNA molecules. We are left with the question of whether miRNAs are truly different from siRNAs or whether our current understanding fails to functionally distinguish these two classes of small silencing RNAs under physiological conditions.

**Roles of miRNAs in Plants**

In plants, miRNAs pair to mRNAs with near-perfect complementarity, and it can direct cleavage of targets mRNAs. Plant miRNAs play an important role in many aspects, including development, and plant diseases.

DCL1 (Dicer-like enzyme 1) is an important enzyme that is involved in miRNA maturation (Park et al, 2002; Reinhart et al, 2002; Papp et al, 2003; Kurihara et al,
Loss-of-function of the DCL1 gene reduced the expression level of mature miRNAs and consequently caused many developmental abnormalities, including arrested embryos at early stages, altered leaf shape and morphology, delayed floral transition, and female sterility (Liu et al, 2005; Kurihara et al, 2006; Park et al, 2002; Reinhart et al, 2002). HASTY is an ortholog of Exportin 5, which transports pre-miRNA from the nucleus to the cytoplasm. Loss-of-function of the HASTY gene also caused developmental abnormalities, such as disrupting leaf shape, flower morphology, accelerating phase change, and reducing fertility (Bollman et al, 2003). These findings indicate that plant miRNAs play an important role in development process at different organ level, including roots, stems, shoots, and flowers.

The plant miRNAs have a remarkable tendency for targeting transcription factor gene families, particularly those with known or suspected roles in plant development (Rhoades et al, 2002) (Table 1). MiRNAs control leaf development by regulating the expression of class-3 homeodomain leucine zipper (HD-ZIP) transcription factor genes, which control leaf asymmetry pattern (Juarez et al, 2004). Mutations in these genes results in morphogenesis of leaf and vascular bundles in the stem (McConnell et al, 2001; Emery et al, 2003). Experiments demonstrated that these transcription factor genes are the targets of and regulated by miR-165 and miR-166 (Emery et al, 2003; Bao et al, 2004; Bowman, 2004; Juarez et al, 2004; Mallory et al, 2004b; Zhong et al, 2004; Kim et al, 2005; Williams et al, 2005b; Ko et al, 2006). Abnormal expression of miR-165 and miR-166 resulted in leaf developmental abnormalities in many plant species, including Arabidopsis and corn (Juarez et al, 2004). MiRNAs control apical meristem development by targeting several members of the NAC (NAM/ATAF/CUC)-domain transcription factors that are important for embryogenic, floral, shoot and root development (Aida et al, 1997; Takada et al, 2001; Hibara et al, 2003). Abnormal expression of miR-164 resulted in abnormal expression of NAC-domain transcription factor genes, consequently caused developmental abnormalities, including shoot and root development (Laufs et al, 2004; Mallory et al,
Many plant miRNAs function during cellular differentiation by mediating the degradation of key regulatory gene transcripts in specific daughter cell lineages (Rhoades et al, 2002). During differentiation, certain genes specifying a less differentiated state need to be turned off by repressing transcription. A gene is not fully turned off until its mRNA stops translating. To make it happen more quickly, miRNA in the differentiating cell can specify the cleavage of that mRNA. This could result in rapid cell differentiation without having to depend on regulatory genes having constitutively unstable messages (Bartel 2004).

MiRNAs are also involved in plant diseases. Some of these miRNAs may get involved in virus-induced gene silencing. Helper component-proteinase (HC-pro), p19, p21, and p69, are unrelated viral suppressors of gene silencing and they play important roles in the virus response to plant antiviral silencing response (Plisson et al, 2003; Chapman et al, 2004). Several lines of evidence indicate that miRNAs are related to the activity of these viral suppressors (Kasschau et al, 2003; Chapman et al, 2004; Chen et al, 2004; Llave 2004). HC-pro inhibited the expression level and activity of mir-171, and caused miR-171 mediated developmental deficiency (Kasschau et al, 2003). P69 enhanced the expression and the activity of miRNAs, and caused rapid degradation of miRNA mediated mRNAs, and consequently enhanced plant resistance to pathogens (Chen et al, 2004). Bacterial flagellin-derived peptide induced an over expression of miR-933 in Arabidopsis (Navarro et al, 2006). MiR-933 negatively regulated F-box auxin receptors (TIR1, AFB1, AFB2, and AFB3), and resulted in inhibited bacteria Pseudomonas syringae growth and increased plant resistance to pathogens (Navarro et al, 2006).
Roles of miRNAs in animals

In animals, it is more difficult to identify miRNAs targets than in plants because in animals there are far fewer mRNAs with near-perfect complementarity to miRNAs. This makes the analysis much more prone to false positives. Evolutionary conservation was used as a criterion for target identification, but it could not be used as a tool to independently validate the targets (Bartel 2004). However, experimental evidence supports a majority of the predictions (Table 1). The function of animal miRNAs has been studied by several approaches. As we know, Dicer and Argonaute proteins are two important enzymes in biogenesis and functions of miRNAs (Hutvagner et al, 2001). Loss-of-function of these two enzymes decreases the expression of global miRNAs and can be used to study the global functions of miRNAs (Meister et al, 2004; Karube et al, 2005). Knockdown or knockdown or overexpression of specific miRNAs is a good approach to investigate the specific function of a unique miRNA (Hutvagner et al, 2004; Meister et al, 2004a; Lee et al, 2005). Recently developed miRNA microarray technology and miRNA specific real-time PCR also provide useful information on miRNA functions (Miska et al, 2004; Bartel et al, 2005; Liang et al, 2005).

MiRNAs regulate differentiation

Two founding member of the class of miRNAs, also the best-studied miRNAs, lin-4 and let-7, regulate differentiation in C. elegans (Lee et al, 1993; Wightman et al, 1993; Reinhart et al, 2000). Loss-of-function of lin-4 and let-7 miRNAs results in abnormal differentiation. Lin-4 controls worm development at an early stage at the first larval stage (L1) and controls worm developmental transition from the L1 stage to the L2 stage by negatively regulating the expression of two genes lin-14 and lin-28 (Lee et al, 1993). Let-7 controls worm development at a late stage and controls worm developmental transition from the L2 stage to L3 stage by negatively regulating the...
expression of another two genes lin-41 and hbl-1 (Reinhart et al, 2000).

**MiRNAs regulate development**

MiRNAs regulate animal development in multiple tissues and at multiple developmental stages. Evidence indicates that miRNAs are important for the development of almost all animal tissues, including stem cells, embryo, brain, heart, limb, liver and other tissues (Lagos-Quintana et al, 2002; Houbaviy et al, 2003; Suh et al, 2004; Lee et al, 2005; Biemar et al, 2005; Schulman et al, 2005; Wienholds et al, 2005; Yang et al, 2005; Krichevsky et al, 2003; Miska et al, 2004; Nelson et al, 2005; Wu et al, 2005; Schubert, 2005; Hornstein et al, 2005; Murchison et al, 2005) (Table 2). For example, in zebrafish, maternal-zygotic dicer (MZdicer) mutant undergo axis formation and differentiate multiple cell type but display abnormal morphogenesis during gastrulation, brain formation, somatogenesis and heart development (Giraldez et al, 2005). HOX is an important group of genes in animal development, and it is negatively regulated by miR-196 and miR-181 (Yekta et al, 2004, Naguibneva et al, 2006). Abnormal expression of these miRNAs causes abnormal expression of HOX, and results in animal developmental abnormality (Mansfield et al, 2004; Yekta et al, 2004; Guenther et al, 2005; Naguibneva et al, 2006). Recent research found that a cardiac-specific miRNA (miR-208) is required for cardiomyocyte hypertrophy, fibrosis, and expression of bMHC (β-myosin heavy chain, the primary contractile protein of the heart) in response to stress and hypothyroidism, which results from down-regulated α MHC and up-regulated β MHC by miR-208 (Rooij et al, 2007).
<table>
<thead>
<tr>
<th>miRNA</th>
<th>Target genes</th>
<th>Biological role of miRNA/target gene</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nematodes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lin-4</td>
<td>Ce lin-14 probable transcription factor</td>
<td>Timing of early larval developmental transitions</td>
</tr>
<tr>
<td></td>
<td>Ce lin-28 cold shock domain protein</td>
<td>Timing of early larval developmental transitions</td>
</tr>
<tr>
<td>let-7</td>
<td>Ce lin-41 probable RNA-binding protein</td>
<td>Timing of late larval developmental transitions</td>
</tr>
<tr>
<td></td>
<td>Ce hbl-1 transcription factor</td>
<td>Timing of late larval developmental transitions</td>
</tr>
<tr>
<td>isy-6</td>
<td>Ce cog-1 transcription factor</td>
<td>Left/right asymmetry of chemoreceptor expression</td>
</tr>
<tr>
<td><strong>Insects</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bantam miRNA</td>
<td>Dm hid pro-apoptotic protein</td>
<td>Apoptosis and growth control during development</td>
</tr>
<tr>
<td>miR-14</td>
<td>Dm grim antagonist of caspase inhibitor</td>
<td>Promotes apoptosis</td>
</tr>
<tr>
<td></td>
<td>Dm reaper antagonist of caspase inhibitor</td>
<td>Promotes apoptosis</td>
</tr>
<tr>
<td></td>
<td>Dm sickle antagonist of caspase inhibitor</td>
<td>Promotes apoptosis</td>
</tr>
<tr>
<td>miR-7</td>
<td>Dm HLHm3 basic HLH transcriptional repressor</td>
<td>Interprets Notch-mediated decisions in neuronal development</td>
</tr>
<tr>
<td></td>
<td>Dm hairy basic HLH transcriptional repressor</td>
<td>Interprets Notch-mediated decisions in neuronal development</td>
</tr>
<tr>
<td></td>
<td>Dm m4 Brd family protein</td>
<td>Interprets Notch-mediated decisions in neuronal development</td>
</tr>
<tr>
<td><strong>Mammals</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-181</td>
<td>unknown</td>
<td>Hematopoietic differentiation</td>
</tr>
<tr>
<td>miR-1</td>
<td>Hs Brain-derived neurotropic factor (BDNF)</td>
<td>Growth factor, neuronal development</td>
</tr>
<tr>
<td></td>
<td>Hs Glucose-6-phosphate dehydrogenase (G6PD)</td>
<td>Oxidoative stress resistance</td>
</tr>
<tr>
<td>miR-19a</td>
<td>Hs Ptdna (3,4,5) P3 phosphatase (PTEN)</td>
<td>Tumor suppressor gene</td>
</tr>
<tr>
<td>miR-23a</td>
<td>Hs Stromal cell-derived factor 1 (SDF-1)</td>
<td>Growth and localization of hematopoletic progenitor cells</td>
</tr>
<tr>
<td></td>
<td>Hs BRN-3β POU-domain transcription factor</td>
<td>Neuronal development</td>
</tr>
<tr>
<td>miR-26a</td>
<td>Hs SMAD-1 transcriptional co-modulator</td>
<td>Regulates TGF-dependent gene expression</td>
</tr>
<tr>
<td>miR-34</td>
<td>Hs Delta 1 transmembrane protein</td>
<td>Activates Notch during cell-fate decisions</td>
</tr>
<tr>
<td></td>
<td>Hs Notch 1 transmembrane receptor for Delta</td>
<td>Cell-fate decisions during development</td>
</tr>
<tr>
<td>miR-101</td>
<td>Hs ENX-1 polycomb gene</td>
<td>Proliferation of hematopoetic cells and other gene regulation</td>
</tr>
<tr>
<td></td>
<td>Hs N-MYC basic HLH transcription factor</td>
<td>Proto-oncogene; cell differentiation and proliferation</td>
</tr>
<tr>
<td>miR-130</td>
<td>Hs Macrophage colony stimulating factor-1</td>
<td>Mononuclear phagocytic lineage regulation</td>
</tr>
<tr>
<td><strong>Plants</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-165/166</td>
<td>At REV and related transcription factors</td>
<td>Axial meristem initiation and leaf development</td>
</tr>
<tr>
<td>miR-172</td>
<td>At AP2 and related transcription factors</td>
<td>Flower development; timing transition to flowering</td>
</tr>
<tr>
<td>miR-JAW</td>
<td>At TCP4 and related transcription factors</td>
<td>Leaf development, embryonic patterning</td>
</tr>
<tr>
<td>miR-159</td>
<td>At MYB33 and related transcription factors</td>
<td>Leaf development</td>
</tr>
<tr>
<td>miR-170/171</td>
<td>At SCL6-3,4 &amp; related transcription factors</td>
<td>Related to genes for root radical patterning</td>
</tr>
<tr>
<td>miR-156/157</td>
<td>At SPL2 &amp; related transcription factors</td>
<td>Related to genes for floral meristem identity</td>
</tr>
<tr>
<td>miR-160</td>
<td>At ARF10, ARF17 &amp; related transcription factors</td>
<td>Related to genes for auxin response &amp; development</td>
</tr>
<tr>
<td>miR-167</td>
<td>At ARF8 &amp; ARF6 transcription factors</td>
<td>Related to genes for auxin response &amp; development</td>
</tr>
<tr>
<td>miR-164</td>
<td>At CUC1, CUC2 &amp; related transcription factors</td>
<td>Shoot apical meristem formation &amp; organ separation</td>
</tr>
<tr>
<td>miR-169</td>
<td>At CBF-HAP2 DNA-binding proteins</td>
<td>unknown</td>
</tr>
<tr>
<td>miR-162</td>
<td>At DCL1 Dicer-like RNase 3</td>
<td>miRNA biogenesis</td>
</tr>
</tbody>
</table>

Table 1: MiRNAs and their functions (modified from Bartel 2004)
Species abbreviation: Ce: Caenorhabditis elegans; Dm: Drosophila melanogaster; Hs: human; At: Arabidopsis thaliana


**MiRNAs regulate cell death and fat metabolism**

The Drosophila miRNA miR-14 suppresses cell death and is required for normal fat metabolism. Loss-of-function of miR-14 enhances Reaper-dependent cell death, whereas ectopic expression suppresses cell death induced by multiple stimuli. MiR-14 also regulates fat metabolism. Deletion of miR-14 results in animals with increased levels of triacylglycerol and diacylglycerol, whereas increases in miR-14 copy number have the opposite effect (Xu et al, 2003).

<table>
<thead>
<tr>
<th>Expression pattern</th>
<th>MiRNAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enriched in brain</td>
<td>miR-12a, miR-125b, miR-128, miR-132, miR-139, miR-7, miR-9, miR-124a, miR-124b, miR-135, miR-153, miR-149, miR-183, miR-190, miR-219</td>
</tr>
<tr>
<td>Enriched in lung</td>
<td>miR-18, miR-19a, miR-24, miR-32, miR-130, miR-213, miR-20, miR-141, miR-193, miR-200b</td>
</tr>
<tr>
<td>Enriched in spleen</td>
<td>miR-99a, miR-127, miR-142a, miR-142s, miR-151, miR-189, miR-212</td>
</tr>
<tr>
<td>Enriched in liver</td>
<td>miR-122a, miR-152, miR-194, miR-199, miR-215</td>
</tr>
<tr>
<td>Enriched in heart</td>
<td>miR-1b, miR-1d, miR-133, miR-206, miR-208, miR-143</td>
</tr>
<tr>
<td>Enriched in kidney</td>
<td>miR-30b, miR-30c, miR-18, miR-20, miR-24, miR-32, miR-141, miR-193, miR-200b</td>
</tr>
<tr>
<td>Enriched in haematopoetic tissues</td>
<td>miR-181, miR-223, miR-142</td>
</tr>
<tr>
<td>Enriched in β-cells of the pancreas</td>
<td>miR-375, miR-125b</td>
</tr>
<tr>
<td>Ubiquitously expressed</td>
<td>miR-16, miR-26a, miR-27a, miR-143a, miR-21, let-7a, miR-7b, miR-30b, miR-30c</td>
</tr>
</tbody>
</table>

Table 2: Tissue-specific miRNA expression signature (Houbaviy et al, 2003; krichevsky et al, 2003; Liu et al, 2004; Sempere et al, 2004; Poy et al, 2005)
MiRNAs are involved in a broad spectrum of human disease. Fragile X mental retardation (FXMR), a neurological disease in which miRNAs or their processing machinery have been induced, is caused by absence or mutation of the fragile X mental retardation protein. Experimental results from Drosophila melanogaster indicate that FXMR may be a part of RISC (Ishizuka et al, 2002).


MiRNAs maybe also involved in virus-related or induced disease and immune defense (Lecellier et al, 2005). MiR-32 mediates antiviral defense in human cells, and regulate primate foamy virus type I (PFV-1) proliferation (Lecellier et al, 2005). MiR-155 plays a key role in the homeostasis and function of the immune system, and it is required for the function of B and T lymphocytes and dendritic cells (Rodriguez et al, 2007).

One of the biggest progresses on miRNAs was the finding that miRNAs play an important role in cancer pathogenesis. About 50% of miRNA genes are localized in cancer-associated genomic regions or in fragile sites (Calin et al, 2004). The prediction number of miRNAs in the human genome is as many as 1000 (1-5% of the predicted genes in the genome). MiRNAs may act as a novel class of oncogenes or tumor suppressor genes (figure 7) (Box 2). They relate to many common and important cancers, including cancers in lung, brain, breast, blood, liver, colon, lymphomas, thyroid, and testicular germ cell (Zhang et al, 2006f) (Table 3).
Box 2: Oncogenes and tumor suppressor genes

Cancer-critical genes are grouped into two broad classes, according to whether the cancer risk arises from too much activity of the gene product, or too little. Genes of the first class, for which a gain-of-function mutation drives a cell toward cancer, are called *proto-oncogenes*; their mutant, overactive forms are called *oncogenes*. Genes of the second class, for which a loss-of-function mutation creates the danger, are called *tumor suppressor genes*.

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Aberrantly regulated miRNAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLL-chronic lymphocytic leukemia</td>
<td>miR-15a and miR-16-1, down regulated in more than 60% of CLL cases</td>
</tr>
<tr>
<td></td>
<td>miR-155/BIC RNA, increased levels</td>
</tr>
<tr>
<td></td>
<td>miR-17-92 cluster, only some members are abnormally expressed</td>
</tr>
<tr>
<td></td>
<td>miR213, miR-183 miR-190, miR-21-1, miRNAs located exactly inside fragile sites</td>
</tr>
<tr>
<td></td>
<td>miR-96, miR-182, miR-183=7q32 group, all members are aberrantly regulated</td>
</tr>
<tr>
<td>CLL-distinguish CLL samples that express unmutated IgVh gene from those</td>
<td>miR-186, miR-132, miR-16-4, miR-102, miR-29c</td>
</tr>
<tr>
<td>that express mutated IgVh gene</td>
<td>miR-15a, miR-195, miR-233 miR-24-1, miR-29b-2, miR-29a-2, miR-16-1, miR-16-2, miR-155, miR-146, miR-221, miR-23b, miR-29c</td>
</tr>
<tr>
<td>CLL-13 miRNAs prognostic group, could discriminate between CLL samples</td>
<td>miR-155, miR-146, miR-221, miR-23b, miR-29c, miR-222, miR-24-2, miR-23a, miR-181a</td>
</tr>
<tr>
<td>that express ZAP-70 and unmutated IgVh and CLL samples that have no</td>
<td></td>
</tr>
<tr>
<td>expression of ZAP and have amutated IgVh</td>
<td></td>
</tr>
<tr>
<td>CLL-9 miRNAs predicting interval from diagnosis to therapy,</td>
<td>miR-155, miR-146, miR-221, miR-23b, miR-29c, miR-222, miR-24-2, miR-23a, miR-181a</td>
</tr>
<tr>
<td>differentiate patients with a short interval from diagnosis from patients</td>
<td></td>
</tr>
<tr>
<td>with a longer intervals</td>
<td></td>
</tr>
<tr>
<td>Diffuse large B cell lymphoma, marginal zone lymphomas, other non-Hodkin</td>
<td>miR-155/BIC RNA, increased levels</td>
</tr>
<tr>
<td>lymphomas and Hodgkin lymphomas</td>
<td></td>
</tr>
<tr>
<td>Aggressive B cell leukemia</td>
<td>miR-152 translocation t(8,17) causes up-regulation of a translocated c-MYC gene</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>miR-10b, miR-125b, miR-145 down-regulated</td>
</tr>
<tr>
<td></td>
<td>miR-21, miR-155, up-regulated</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>Let-7, reduced expression</td>
</tr>
<tr>
<td></td>
<td>miR-155, over-expression</td>
</tr>
<tr>
<td></td>
<td>miR17-92 cluster, over-expression</td>
</tr>
<tr>
<td>glioblastoma</td>
<td>miR-221, miR-21, strongly over-expressed</td>
</tr>
<tr>
<td></td>
<td>miR-128, miR-181a, miR-181b, miR-181c, down-regulated</td>
</tr>
<tr>
<td>Colorectal tumors</td>
<td>miR-143, miR145, down-regulated</td>
</tr>
</tbody>
</table>

Table 3: MiRNAs associated with cancer diseases (Kusenda et al, 2006)
Let-7 regulates lung cancer pathogenesis by negatively regulating the expression of the oncogene RAS (Johnson et al, 2005). RAS is a well-studied oncogene which interferes with p53 pathway in lung cancer, leading to apoptosis, and it has multiple complementarity sites to let-7 in its 3’UTR (Johnson et al, 2005). Thus let-7 inhibited RAS mRNA translation, and inhibited lung tumor cell differentiation and growth (Johnson et al, 2005). A recent study demonstrated that the expression of let-7 was significantly reduced while the expression of RAS was dramatically increased in lung tumor tissues (Johnson et al, 2005). This suggests that miRNA let-7 may function as a tumor suppressor gene in human and other mammals (figure 7).

Evidence indicates that in leukemic cells with a deletion of the locus on chromosome 13 that contains miR-15a and miR-16-1 miRNA genes (Calin et al, 2002), cancer is induced as a result of the down-expression of these miRNAs, in more than 60% of CLL cases (Calin et al, 2002). It was demonstrated that a possible target for miR-15a and miR-16-1, BCL2, is negatively regulated by both miRNAs at a
post-transcriptional level. The expression of both miRNAs is inversely correlated to BCL2 expression in CLL (Cimmino et al, 2005). Moreover, in a leukemic cell line model, BCL2 repression by these miRNAs induces apoptosis (Cimmino et al, 2005) (figure 7). This suggests that deregulation of anti-apoptotic BCL2 in CLL cells could be a key event in cancerogenesis.

MiR-21, the only miRNA overexpressed in all types of cancer analysed, is also overexpressed in glioblastomas and cholangiocarcinomas (Clafre et al, 2005; Chan et al, 2005; Meng et al, 2006). It directly targets the tumor suppressor PTEN in cholangiocarcinoma cells. PTEN encodes a phosphatase that can inhibit growth and survival pathways (Ali et al, 1999). Furthermore, knockdown of miR-21 in glioblastoma cells triggers apoptosis by a caspase-dependent mechanism (Chan et al, 2005). It is suggested that miR-21 is an anti-apoptotic factor and can act as an oncogene.

MiR-155 is a gene overexpressed in various types of B-cell malignancy (Metzler et al, 2004; Eis et al, 2005; Kluiver et al, 2005). Its regulation could be an early event in oncogenesis that needs additional genetic alterations for the development of the fully malignant phenotype (Costinean et al, 2006). There are complex relationships exist between protein-coding genes and miRNAs that affect specific signaling pathways and, probably, direct or indirect interactions exist between miR-155 and some of the other deregulated miRNAs (George et al, 2006).

The miR17-92 cluster is located at 13q31.3, a genomic locus that is amplified in cases of diffuse large B-cell lymphoma, follicular lymphoma, mantel cell lymphoma, primary cutaneous B-cell lymphoma and other tumor types (Knuutila et al, 1998; Ota et al, 2004). O’Donnel et al, 2005 showed that in the human B-cell line P493-6, which overexpress MYC, miRNA members of the miR-17-92 cluster have tumor suppressor activity because their expression decreases the expression of E2F1, and so inhibits MYC-mediated cellular proliferation (O’Donnel et al, 2005). However, a different
perspective is offered by the results from B-cell lymphomas. The same cluster of miRNAs function as a potential oncogene by cooperating with MYC and blocking apoptosis (He et al, 2005). George et al, 2006 suggest that the same miRNA can participate in distinct pathways, having different effects on cell survival, growth and proliferation that are dependent on the cell type and the pattern of gene expression. The combinatorial nature of miRNA-miRNA interaction means that the same miRNA could have different targets and the same mRNA could be targeted by different miRNA in different cell types.

Discussion

A general model for gene post-transcriptional regulation by miRNA holds that perfect complementarity between a miRNA and its target mRNA results in cleavage of the mRNA, partial pairing of a miRNA and its target mRNA leads to translational repression without destabilization of the mRNA. However, evidence showed that regulation by lin-4 and let-7 miRNAs results in target mRNA degradation (Bagga et al, 2005). It was originally reported that the association of lin-14 mRNA with polyribosomes appeared unchanged upon lin-4 mediated repression of protein expression (Olsen et al, 1999), and the lin-28 mRNA levels and polyribosome profile were also not altered when lin-4 blocked lin-28 protein accumulation (Moss et al, 1997; Seggerson et al, 2002). Bagga et al used Northern analyses to compare the expression of full length intact endogenous mRNA targets of miRNA regulation, which leads to the different results.

We think that the mechanism directed by a given miRNA could be dependent on multiple factors, including the composition of mRNA, the expression level of mRNA, and the miRNA cofactors. If the mRNA includes target sites which perfectly base pair to a miRNA, then RNAi is triggered and the mRNA is cleaved by Ago family. If the base pairing is insufficient, we propose that the mechanism may dependent on the
mRNA level which is regulated by other mechanisms. When mRNA level is high, there could be two possibilities. First, the initiation of the translation is inhibited by miRNP interfering initiation factors (figure 6a). Run off of ribosomes results in accumulation of repressed ribosome-free mRNAs in to P-bodies for either storage or decay (Pillai, et al, 2005), which may cause the observation of mRNA level decrease. Second, miRNP recruits the deadenylase complex Ccr4:Not1 and the GW182 protein for mRNA decay (figure 6d). When the situation leads to a low level of mRNA, the mRNA will not destabilized by miRNAs. The translation may be repressed at post-initiation steps, at the elongation, or termination. In this model, mRNAs remain largely associated with ribosomes and thus may not relocate to P-bodies (figure 6b). Alternatively, rapid proteolysis of nascent polypeptide chains results in the decrease of the protein level (figure 6c), while the mRNA level remains unchanged. In summary, miRNA regulates its target mRNA dynamically either by directs mRNA cleavage, or leads to mRNA decay if necessary, consequently decreases the protein level. MiRNAs can down-regulate target mRNA and protein in different manner in multiple situations. Scientists used different experiment techniques could lead to the discrepant results.

Abnormal expression of miRNA usually results in cancer. It was shown that more than half of the known human miRNAs reside in cancer-associated genomic regions that are prone to alteration in cancer cells (Calin et al, 2004). Additionally, alteration of the proteins involved in the biogenesis of miRNA should have dramatic effects on miRNA expression. As affecting important human oncogenic signaling pathway, miRNA can be a new clinical tool in cancer diagnosis and prognosis. Evidence indicates that the miRNA based cancer classifier is much better at establishing the correct diagnosis of the samples than the mRNA classifier (Lu et al, 2005). Profiling a few hundred miRNAs has a much better predictive power for diagnosis than profiling several tens of thousands of mRNA. MicroRNA profiling can also act as a prognostic tool. The identification of new prognostic markers, which correlate with disease evolution, could be a significant advance for the identification of patients that would
benefit from more aggressive therapy. For example, in lung cancer, as illustrated above (table 3, figure 7), the existence of the link between let-7 and Ras expression could have impact on the life expectancy of patient with lung cancer.

**Conclusion**

Since the discovery of miRNAs in C. elegans, remarkable progress has been made in exploring the characterization of this gene family. It has been demonstrated that these small, no-coding RNAs are not only a new class of gene expression regulatory RNAs, but the outlines of the mechanism for their functions in gene regulation have emerged. However, with some exceptions, we know little about the precise mechanisms of the vast majority of miRNAs in regulating gene expression. Furthermore, the relationships between miRNAs, siRNAs and the proteins of the gene regulatory machinery remain to be explained.

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