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2.22 Physiological and Pathological Functions of Mammalian MicroRNAs

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Abbreviations

AIDS	acquired immuno deficiency syndrome
ALL	acute lymphoblastic leukemia
AML	acute myeloid leukemia
bHLH	basic helix–loop–helix
BIC	B cell integration cluster
CLL	chronic lymphocytic leukemia
CMV	cytomegalovirus
DEAD	Asp-Glu-Ala-Asp
DGCR8	DiGeorge syndrome critical region gene 8
dsRBD	dsRNA-binding domain
ES	embryonic stem
EWS	Ewing sarcoma
FMRP	fragile X mental retardation protein
FSTL1	follistatin-like 1
GIST	gastrointestinal stromal tumor
HIV	human immunodeficiency virus
HsAgo2	human Argonaute 2
LMS	leiomyosarcoma
Loqs	loquacious
miRISC	miRNA-induced silencing complex
miRNA	microRNA
miRNP	miRNA ribonucleoprotein
MZdicer	maternal and zygotic dicer
NCBI	National Center for Biotechnology Information
nt	nucleotide
PACT	protein activator of the interferon-induced protein kinase
PAZ	Piwi-Argonaute-Zwille
PIWI	P-element included wimpy testis
pre-miRNA	precursor miRNA
pri-miRNA	primary miRNA
RIIID	RNase III domain
Ran-GTP	RAs-related nuclear protein-Guanosine
RISC	RNA-induced silencing complex
RNAi	RNA interference
siRNA	small interfering RNA
snoRNA	small nucleolar RNA

SRF	serum response factor	**UTR**	untranslated region
TRBP	transactivating response RNA-binding protein	**VIG**	vasa intronic gene

s0005 ## 2.22.1 Introduction

p0005 MicroRNA (miRNA) is a class of small noncoding RNA molecules that regulate gene expression post-transcriptionally. This chapter summarizes recent advances in our understanding of mammalian miRNA functions in physiology and pathology, as well as the implications of these advances in toxicology.

p0010 miRNAs are 19- to 26-nucleotide (nt) RNAs that negatively regulate gene expression in animals and plants (Bartel 2004). The first miRNA, lin-4, was discovered in the nematode *Caenorhabditis elegans* in 1993 (Lee *et al.* 1993; Wightman *et al.* 1993), while the second, let-7, was identified 7 years later (Reinhart *et al.* 2000). Since then, numerous miRNAs have been found in all metazoans (vertebrates, flies, worms, and plants) and in viruses. Over 700 human miRNA genes have been identified, and one report predicts that the number is close to 1000 (Berezikov *et al.* 2005). This makes miRNA genes one of the most abundant classes of regulatory genes in mammals. Recent computational methods for predicting miRNA targets indicate that up to 92% of human genes may be regulated by miRNA (Miranda *et al.* 2006). The physiological and pathological functions of these important regulatory RNA molecules are currently under intense investigation. In this chapter, we summarize recent progress in understanding miRNA biology and the role of miRNAs in human disease development.

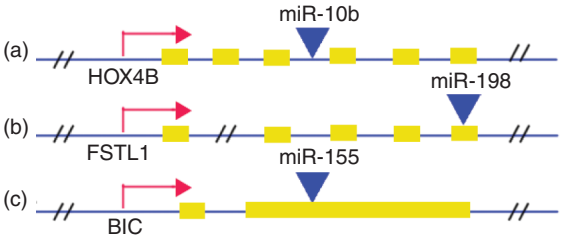
s0010 ## 2.22.2 miRNA Biology

s0015 ### 2.22.2.1 miRNA Genes and Their Genomic Organization

p0015 miRNAs are a large family of 19- to 26-nt single-stranded noncoding RNAs present in nematodes to human (Lagos-Quintana *et al.* 2001; Lee and Ambros 2001). Most miRNAs are evolutionarily conserved in related species and some are even conserved between invertebrates and vertebrates (Lagos-Quintana *et al.* 2001; Lau *et al.* 2001; Lee and Ambros 2001). Currently, about 5400 miRNA genes have been discovered in a variety of organisms. Among these, 564 are from human, 461 are from mouse, and 293 are from rat (reported in the miRBase Sequence Database, Release 10.1, December 2007 (Griffiths-Jones *et al.* 2006)). The miRBase collects known miRNA genes and lists their sequences, genomic positions, and related miRNAs. Most of the miRNA genes have been discovered by direct cloning and sequencing of small RNA fractions extracted from cells and tissue samples (Hirsch *et al.* 2001). The cloned sequences are computationally analyzed for their genomic location and their ability to form a potential hairpin-like precursor miRNA (pre-miRNA) with the sequences flanking the mature RNA ends before they are finally registered as novel miRNA entries in miRBase. Recently, computational approaches have been developed to find new miRNA genes, based on the detection of evolutionarily conserved candidates and pre-miRNA structure (Lindow and Gorodkin 2007; Weber 2005). However, experimental verification of the mature form of miRNAs is required to provide direct evidence that the predicted miRNA genes are authentic.

p0020 The genomic organization of miRNA genes varies: miRNA genes are either within the introns/exons of other nonprotein-coding or protein-coding genes, or they have their own independent transcription units (**Figure 1**). Rodriguez *et al.* (2004) analyzed the positions of known mammalian miRNAs in human and mouse genomes and concluded that ~40% of mammalian miRNAs are within introns in protein-coding genes, ~10% within introns in nonprotein-coding genes, ~10% are within exons, ~30% have uncertain transcriptional units, and the rest are derived from genomic repeats. A recent study of *Xenopus* miRNA genome structure indicated that ~77% of frog miRNA genes are positioned within the introns of RNA polymerase II-transcribed genes, ~7% within pre-mRNA exons, and ~15% within intergenic regions (Tang and Maxwell 2007).

p0025 A distinguishing feature of miRNA genomic organization is that ~50% of miRNAs are polycistronic and are cotranscribed and then subsequently cleaved to yield multiple miRNAs (Baskerville and Bartel 2005; Lagos-Quintana *et al.* 2001; Landgraf *et al.* 2007; Lau *et al.* 2001; Lee *et al.* 2004a; Mourelatos *et al.* 2002; Tang and Maxwell 2007). Clustered miRNAs generally



f0005 **Figure 1** Genomic organization of miRNA genes. miRNA genes are positioning in different genomic loci. (a) The majority of miRNA genes are located within the introns of either protein-coding or nonprotein-coding transcription units. For example, miR-10b is embedded in the intron 4 of HOX4B in mice and humans (Rodriguez *et al*. 2004). (b) A very small number of miRNA genes overlap with exons of known genes and are located mainly in the noncoding 5′- or 3′-UTRs. For example, miR-198 is in exon 11 of FSTL1 (follistatin-like 1) (Cullen 2004). (c) A few miRNA genes are within an exon of a noncoding RNA. This type of miRNA gene could be transcribed independently from their own promoters and enhancers. For example, miR-155 is embedded in exon 2 of the B cell integration cluster (BIC) transcript in human (Rodriguez *et al*. 2004). The genomic location of miRNA genes is shown by a blue triangle, exons are shown by yellow rectangles, and introns are shown by blue lines. The red arrow represents promoters.

possess similar seed sequences and those clusters identified early included two or three miRNA genes. However, more clusters are now known to contain multiple miRNA genes. Examples are the human *mir-17* cluster comprised of seven miRNA genes (He *et al.* 2005b; Ota *et al.* 2004) and the human *mir-302* cluster comprised of five miRNA genes (Landgraf *et al.* 2007). The human *mir-134* cluster has 37 miRNA genes (Landgraf *et al.* 2007), while the human *mir-371* cluster contains 46 miRNA genes (Landgraf *et al.* 2007). Thus, clustering of miRNA genes is greater than originally envisioned (Landgraf *et al.* 2007), and this finding suggests important evolutionary and functional implications. miRNAs within a cluster might work synergistically to affect the same developmental and/or physiological process. For example, miR-1 and miR-133 in the *mir-1–133* cluster are specifically expressed in cardiac and skeletal muscle tissues and they are required for proper heart and skeletal muscle development (Chen *et al.* 2006). The zebrafish *mir-430* cluster is expressed to accelerate the deadenylation and clearance of several hundred maternal transcripts at the onset of zygotic transcription and regulates morphogenesis during early development (Giraldez *et al.* 2005, 2006). In comparison, the miR-143 and miR-145 in the *mir-143–145* cluster is downregulated in colon adenocarcinoma as well as in some B-cell lymphomas (Akao *et al.* 2007a,b; Michael *et al.* 2003). The *mir-17–92* cluster is found to be in a region of DNA that is amplified in human B-cell lymphomas (Ota *et al.* 2004), and genes within the cluster are associated with various human cancers (Esquela-Kerscher and Slack 2006; Hayashita *et al.* 2005; He *et al.* 2005b; Hossain *et al.* 2006; Hwang and Mendell 2006; Thomson *et al.* 2006). The *Xenopus laevis* miR-15 and miR-16 within the *mir-15* cluster restrict the size of the organizer by targeting the Nodal type II receptor *Acvr2a*, and the expression of *mir-15* cluster members is under negative control of Wnt/β-catenin signaling (Martello *et al.* 2007), as human *mir-15* cluster can act as a tumor suppressor and induce leukemia cell apoptosis by targeting *BCL2* (Cimmino *et al.* 2005). This genomic organization of miRNAs in clusters provides a mechanism for coordinated function. Thus, it is reasonable to foretell that clustered miRNAs might compose a dedicated regulatory network that functions by targeting a set of specific mRNAs for a biological process.

2.22.2.2 miRNA Gene Expression

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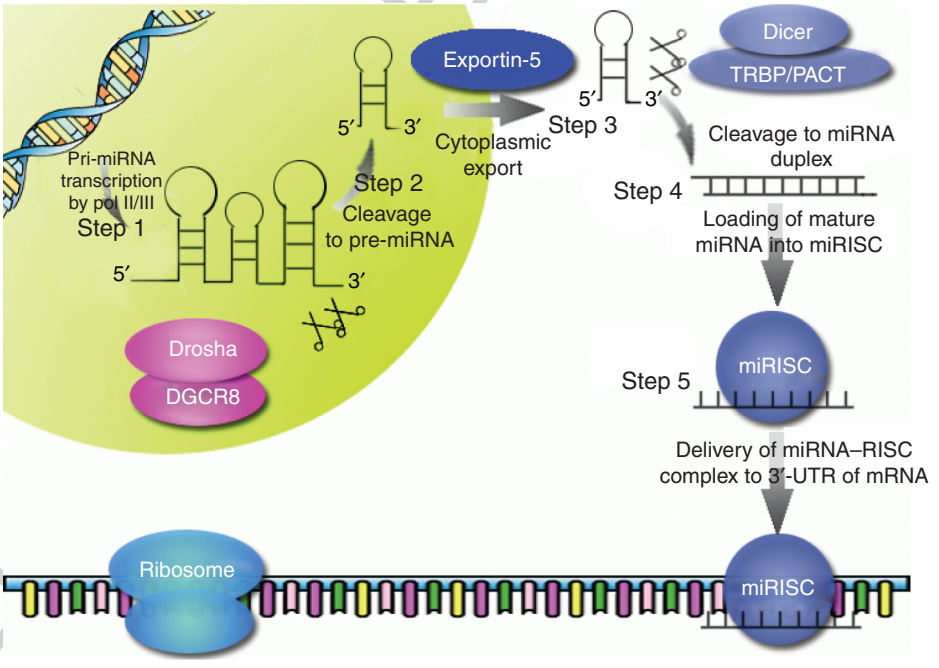
Some miRNA genes have their own promoters and enhancers and therefore they can be expressed independently. Such genes might still be located in the introns of protein-coding genes, but often in an antisense orientation. These miRNA genes are often directly regulated by related transcription factors. For example, the *mir-1–133* gene cluster in vertebrates is specifically expressed in cardiac and skeletal muscle cells. This miRNA gene cluster is believed to be expressed independently with its own promoter and is transcriptionally regulated by myogenic differentiation factors, such as serum response factors (SRFs), MyoD, and Mef2 (Chen *et al.* 2006; Liu *et al.* 2007; Niu *et al.* 2007; Rao *et al.* 2006; Zhao and Srivastava 2007; Zhao *et al.* 2005). SRF activates transcription of the *mir-1–133* genes, and SRF knockout mice lack endogenous miR-1 expression in the heart (Niu *et al.* 2007; Zhao and Srivastava 2007; Zhao *et al.* 2005). In skeletal muscle, MyoD and Mef2 directly regulate miR-1 and miR-133 expression (Chen *et al.* 2006; Liu *et al.* 2007; Rao *et al.* 2006). Transcription factor binding sites are conserved in fruit flies and worms, where an SRF-like site is essential for cardiac expression and the basic helix–loop–helix (bHLH) transcription factors twist and mef2 regulate somatic muscle expression of miR-1 (Kwon *et al.* 2005; Sokol and Ambros 2005; Zhao and Srivastava 2007). Another muscle-specific miRNA gene, *mir-206*, is also directly activated by MyoD (Rosenberg *et al.* 2006).

p0035 miRNA genes are often coexpressed with their host protein-coding genes (Baskerville and Bartel 2005). The majority of mammalian miRNA genes are located within the intronic regions of either coding or non-coding genes and are transcribed as part of their host transcription units (Rodriguez *et al.* 2004). As is the case with intronic small nucleolar RNAs (snoRNAs) (Filipowicz and Pogacic 2002), it has generally been assumed that Drosha/DGCR8 recognizes miRNA-containing introns excised during the splicing reaction as pre-miRNAs and thus both mRNA and miRNA can be coexpressed from a single primary transcript. However, a recent study indicates that intronic miRNAs can also be processed by Drosha/DGCR8 from unspliced pre-mRNAs (Kim and Kim 2007).

p0040 In most cases, polycistronic miRNAs display the same expression pattern (Baskerville and Bartel 2005); however, relative levels of miRNAs within a single cluster might be regulated in a developmental and homeostatic manner. Recently, Yu *et al.* (2006) analyzed the expression of miRNA clusters in human leukemia cell lines and found that 39 miRNA clusters had members that displayed the same expression pattern, whereas 12 clusters had members with different levels of expression. Boggs *et al.* (2007) quantified the expression levels of seven miRNAs in the *mir-17–92* canine cluster and found that not only the expression of the cluster varies in different tissues, but also the levels of individual miRNA within the cluster vary in the same tissue. For example, the expression of miR-17-3p and miR-17-5p is relatively low compared to other miRNAs in the cluster (Boggs *et al.* 2007). In addition, although the human miR-127 gene is located within a cluster with miR-136 and eight other miRNAs on chromosome 14q32.31 (Altuvia *et al.* 2005; Landgraf *et al.* 2007), miR-127 and miR-136 have different expression patterns in human cancers (Iorio *et al.* 2005; Lu *et al.* 2005), suggesting that the expression of miR-127 and miR-136 is differentially regulated, at least in certain types of human cancers. Further studies revealed that miR-127 is subject to epigenetic silencing in cancer cells, but is expressed from an miRNA cluster in normal fibroblasts (Saito *et al.* 2006).

2.22.2.3 miRNA Biogenesis s0025

p0045 In animals, at least five steps are required for miRNA maturation, as shown in **Figure 2**. First, miRNA genes are transcribed by RNA polymerase II or III (Borchert *et al.* 2006; Lee *et al.* 2004a), generating



f0010 **Figure 2** miRNA biogenesis pathway. Step 1: miRNA genes are transcribed by RNA polymerase II and/or III as long primary miRNAs (pri-miRNAs). Step 2: pri-miRNAs are recognized and cleaved by the nuclear Drosha/DGCR8 complex in the nucleus, generating pre-miRNAs. Step 3: pre-miRNAs are transported to the cytoplasm by Exportin-5. Step 4: pre-miRNAs are recognized and processed by Dicer, yielding an imperfect miRNA duplex. Step 5: miRNA duplex is unwound, and the mature miRNAs are asymmetrically incorporated into an effector complex called the miRNA-induced silencing complex (miRISC). Then the miRNA/RISC complex represses protein translation by binding to sequences in the 3′-UTR of specific mRNAs. The exact mechanism of translation repression is still undefined.

primary transcripts (pri-miRNAs) as unclustered monocistronic or clustered polycistronic RNAs, which may be several hundred nucleotides to several thousand nucleotides long. Second, pri-miRNAs are recognized and cleaved by the nuclear Drosha complex that consists of ribonuclease (RNase) III family member Drosha and its cofactors (Denli *et al.* 2004; Gregory *et al.* 2004; Han *et al.* 2004). This cleavage occurs in the nucleus and liberates 60–70 nt stem-loop miRNA intermediates, known as pre-miRNAs, from pri-miRNAs (Lee *et al.* 2002; Zeng *et al.* 2003). Like all RNase III enzymes, Drosha leaves 2 nt 3′ overhangs and 5′ phosphates in the products (Carmell and Hannon 2004). Third, pre-miRNAs are transported to the cytoplasm by the nuclear export factor Exportin-5 in a RAs-related nuclear protein-guanosine triphosphate (Ran-GTP)-dependent manner (Lund *et al.* 2004; Yi *et al.* 2003; Zeng and Cullen 2006). Fourth, pre-miRNAs are recognized and processed by the cytoplasmic RNase III endonuclease Dicer, yielding a 20–25 nt imperfect miRNA duplex (miRNA*:miRNA) with characteristic 5′ phosphates and 2 nt 3′ overhangs. Dicer alone is sufficient for processing pre-miRNAs and double-stranded RNAs (dsRNAs), while some dsRNA-binding partners are required for efficient miRNA/small interfering RNA (siRNA) production, such as the human immunodeficiency virus (HIV) transactivating response RNA-binding protein (TRBP) and/or the protein activator of the interferon-induced protein kinase (PACT) in humans (Chendrimada *et al.* 2005; Kok *et al.* 2007; Lee *et al.* 2006) or Loquacious (Loqs) in flies (Forstemann *et al.* 2005; Jiang *et al.* 2005; Saito *et al.* 2005). Finally, miRNA*:miRNA duplexes are unwound, and the mature miRNAs are asymmetrically incorporated into an effector complex called miRNA ribonucleoprotein (miRNP) particles or miRNA-induced silencing complex (miRISC). This complex consists of Argonaute family proteins and the miRNA and regulates gene expression by mRNA degradation or translational repression. The miRNA* is quickly degraded (Chendrimada *et al.* 2005; Gregory *et al.* 2005; Maniataki and Mourelatos 2005; Martinez *et al.* 2002). It appears that strand selection might be determined by the relative thermodynamic stability of the miRNA duplex ends (Khvorova *et al.* 2003; Schwarz *et al.* 2003).

p0050 Two members of the RNase III family, Dicer and Drosha, play crucial roles in the RNA silencing pathways (Carmell and Hannon 2004). This family of enzymes has major functions in RNA processing (Robertson *et al.* 1968), posttranscriptional gene expression control (Oppenheim *et al.* 1993; Wu *et al.* 2000), and defense against viral infection (Saleh *et al.* 2004; van Rij and Andino 2006). Compared to their bacterial counterparts, Drosha and Dicer are much bigger and more complicated. Bacterial RNase III is composed of an RNase III domain (RIIID) followed by a dsRNA-binding domain (dsRBD) (Robertson *et al.* 1968), whereas Drosha possesses an extended N-terminus that contains a proline-rich region and a serine/arginine-rich region of unknown function and two RIIIDs and a dsRBD in the C-terminus (Blencowe *et al.* 1999; Lee *et al.* 2003). The Dicer protein has a long N-terminus containing an RNA helicase/adenosine triphosphatase (ATPase) domain, a DUF283 domain, and a Piwi-Argonaute-Zwille (PAZ) domain apart from tandem RIIIDs and a dsRBD (Bernstein *et al.* 2001; Provost *et al.* 2002; Zhang *et al.* 2002). The DUF283 and PAZ domains, but not the RNA helicase/ATPase domain, are required for *in vitro* Dicer activity (Ye *et al.* 2007). The PAZ domain is also found in Argonaute family proteins that have been implicated in RNA interference (RNAi) function. In fact, PAZ was defined from the three Argonaute family proteins Piwi, Argonaute, and Zwille (Bernstein *et al.* 2001; Cerutti *et al.* 2000). The domain appears to bind to the end of dsRNA to mark a specific distance from the sites cleaved by either Argonaute's slicer activity or Dicer's dicing activity (Lingel *et al.* 2004; Macrae *et al.* 2006; Song *et al.* 2003; Yan *et al.* 2003). Similar to Dicer, bacterial RNase III, containing a single RIIID, functions as a dimer and the dimeric RIIIDs form an intermolecular active center that cleaves two strands of dsRNA simultaneously (Gan *et al.* 2006), while human Dicer is suggested to work as a monomer with its tandem RIIIDs forming one processing center and cleaving the opposite strand of dsRNA (Zhang *et al.* 2004). This model is supported by the X-ray crystal structure of *Giardia* Dicer that carries only a PAZ domain followed by tandem RIIIDs (Macrae *et al.* 2006). Drosha also appears to form an intramolecular dimer of two RIIIDs with two closely placed catalytic sites to cleave the 3′ and 5′ strands of the stem, respectively (Han *et al.* 2004).

Dicer is essential for miRNA biogenesis (Jiang p0055
et al. 2005; Lee *et al.* 2004b; Saito *et al.* 2005). Loss of function of *dcr-1* disrupted miRNA processing and blocked the miRNA pathway of gene expression in flies (Lee *et al.* 2004b; Liu *et al.* 2007). Zebrafish embryos deficient for maternal and zygotic Dicer (MZdicer) activity cannot generate mature miRNAs (Giraldez *et al.* 2005; Wienholds *et al.* 2003). In addition, Dicer is also essential for the processing of dsRNAs into siRNAs and for producing siRNAs for

RNAi pathways (Bernstein *et al.* 2001; Ketting *et al.* 2001). Moreover, biochemical analysis revealed that Dicer interacts directly with Argonaute family proteins, which are the core components of the RNA-induced silencing complex (RISC), implicating a role of loading of small RNA molecules with Argonaute protein (Meister *et al.* 2005). Indeed, Dicer, as well as its cofactors TRBP and PACT in humans (Chendrimada *et al.* 2005; Kok *et al.* 2007; Lee *et al.* 2006) or Loqs and R2D2 in flies (Forstemann *et al.* 2005; Jiang *et al.* 2005; Liu *et al.* 2003; Saito *et al.* 2005; Tomari *et al.* 2004), is required for the assembly of the effector miRNA-containing miRISC complexes or siRNA-containing siRNA-induced silencing complex (siRISC). Probably due to its indispensable roles in both miRNA and siRNA production, loss of function of *dicer* results in animal death and developmental abnormalities (for details, see Section 2.22.2.5) (Bernstein *et al.* 2003; Harris *et al.* 2006; Lee *et al.* 2004b; Muljo *et al.* 2005; Wienholds *et al.* 2003; Yang *et al.* 2005).

p0060 Drosha is crucial to the precise processing of pri-miRNA that can be hundreds to thousands of nucleotides long into 60–70 nt pre-miRNA intermediates. It appears that Drosha, on its own, is not sufficient to accomplish pri-miRNA cleavage and requires some RNA-associated factors to carry out the processing. Two types of functional Drosha complexes have been biochemically identified: the smaller one is a di-subunit complex consisting of Drosha and a dsRNA-binding protein encoded by the *DiGeorge syndrome critical region gene 8* (DGCR8) in humans (known as Pasha in *Drosophila melanogaster* and *C. elegans*) (Denli *et al.* 2004; Gregory *et al.* 2004; Han *et al.* 2004; Landthaler *et al.* 2004); the larger one contains Drosha and nearly 20 polypeptides. These polypeptides represent multiple classes of RNA-associated proteins, including an RNA helicase, DDX17/P72, a heterogeneous nuclear ribonucleoprotein, hnRNPM4, and the Ewing sarcoma (EWS) family of proteins containing an RNA recognition motif and a zinc-finger domain (Gregory *et al.* 2004). The Drosha/DGCR8 di-subunit complex appears to be necessary and sufficient for processing pri-miRNA into pre-miRNA. However, a recent report suggested that p68–p72 ATP-dependent Asp-Glu-Ala-Asp (DEAD)-box RNA helicases are required for recognition of a subset of pri-miRNAs in mouse Drosha-mediated processing (Fukuda *et al.* 2007), suggesting that generation of miRNA intermediates from pri-miRNA with diverse structures might require additional RNA-associated factors for Drosha-mediated processing. In addition, Drosha complexes are also required for processing 12S precursor ribosomal RNA (pre-rRNA) to 5.8S rRNA and may also be involved in the cleavage of 32S pre-rRNA to 12S pre-rRNA and 18S rRNA (Fukuda *et al.* 2007; Lee *et al.* 2003; Wu *et al.* 2000). Remarkably, a Drosha-bypass alternative miRNA biogenesis pathway was recently identified in flies and nematodes, in which Drosha is not required for the biogenesis of some miRNAs from a type of intronic pre-miRNAs called mitrons. For these mitrons, splicing by the spliceosome, rather than Drosha, defined the mirtronic pre-miRNAs. Mirtronic pre-miRNAs are of comparable size to pre-miRNAs after they have been excised by the splicing reaction and the intronic pre-miRNAs are directly exported from the nucleus to the cytoplasm by Exportin-5 and cleaved by Dicer-1, that is, Loqs. As expected, knockdown of *drosha* had little effect on mature mirtronic miRNA accumulation and a modest effect on mirtronic pre-miRNAs, while knockdown of *dicer-1* or its partner, *loqs*, increased the ratio of mirtronic pre-miRNA to mature mirtronic miRNA (Ruby *et al.* 2007).

p0065 Most recently, Diederichs and Haber (2007) have reported that the RISC slicer Argonaute 2 cleaves the pre-miRNA to an additional processing intermediate, termed Ago2-cleaved precursor miRNA or ac-pre-miRNA, indicating that Argonaute plays a vital role in miRNA biogenesis.

2.22.2.4 Mechanisms of miRNA-Mediated Gene Regulation

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p0070 After production by Dicer, miRNAs are directly assembled into effector miRISC complexes to direct sequence-specific translational repression and/or degradation of cognate mRNA. The essential core components of the RISC are members of the Argonaute family of proteins, which contain conserved PAZ and P-element induced wimpy testis (PIWI) domains. The PAZ domain forms a specific binding module for the characteristic 2 nt 3′ overhangs generated by RNase III-type enzymes such as Dicer, and the PIWI domain folds similar to RNase H and interacts with the miRNA/siRNA guide strand at its 5′ end (Filipowicz 2005). Of the human Argonautes 1–4, only RISC-containing human Argonaute 2 (HsAgo2) is cleavage-competent and is responsible for mRNA cleavage in RISC (Liu *et al.* 2004; Meister *et al.* 2004). Additional protein components in RISC have been identified in various organisms, such as the vasa

intronic gene (VIG), Tudor-SN, the fragile X mental retardation protein (FMRP), gemin 4 and the DEAD-box RNA helicase gemin, MOV10, another putative DEAD-box helicase, TNRC6B52, and PACT. The precise order of RISC assembly, the proteins and factors involved, as well as the function of additional proteins within the complex remain unclear.

p0075 Previous studies have suggested that *Drosophila* Ago1 and Ago2 are restricted to the miRNA and siRNA pathways, respectively (Okamura *et al.* 2004). Such restriction of each class of small RNA to a distinct Argonaute complex could occur because miRNAs and siRNAs in flies are separately produced by Dicer-1 and Dicer-2, respectively (Jiang *et al.* 2005; Lee *et al.* 2004b; Liu *et al.* 2003; Saito *et al.* 2005). Since the two members of the *Drosophila* Ago proteins are functionally specialized, Ago2 with slicer activity in siRISC mediates cleavage of the target mRNA, while Ago1 with inefficient nuclease in miRISC represses the translation of an mRNA containing partially complementary miRNA-binding sites in its 3′-untranslated region (UTR). Specifically, the Ago proteins in the RISC complexes determine the regulatory mechanism that the guided RNAs use to silence their targets. However, most plant miRNAs and several animal miRNAs do guide cleavage of their target mRNAs in a manner similar to that of siRNAs. Remarkably, recent studies indicated that miRNAs and siRNAs in flies participate in a common sorting step that partitions them into Ago1- or Ago2-containing effector complexes (Forstemann *et al.* 2007; Tomari *et al.* 2007), providing the feasibility of miRNA-mediated cleavage.

p0080 In fact, most animal miRNAs mediate translational repression rather than cleavage of their target mRNAs. It is believed, at least in part, that the degree of complementarity between an miRNA and its target determines the regulatory mechanism: miRNAs with nearly perfect base pairing with their corresponding targets are most likely to guide endonucleolytic cleavage of their regulatory targets, while those lacking sufficient complementarity promote sequence-specific repression of mRNA translation and/or accelerate mRNA decay. Interestingly, extensive base pairing between miRNA and mRNA is not always sufficient to induce cleavage, suggesting that there can be additional requirements for an RISC complex to catalyze endonucleolytic cleavage (Chen *et al.* 2004). Only 6–7 nt, usually nucleotides 2–8, the so-called 'seed sequence' of miRNAs, have been shown to be critical and, in some cases, sufficient for target recognition and silencing (Doench and Sharp 2004; Lewis *et al.* 2005). However, data on the exact mechanism of translational repression are unclear due to the fact that both the initiation and elongation steps of translation are thought to be affected. Moreover, miRNAs were found to direct rapid deadenylation and decay of their target mRNAs (Wu *et al.* 2006), and *in vitro* studies showed that a 7-methyl G cap and a polyA tail are required for translational gene repression of the targets by miRNAs, whereas increasing polyA tail length alone can increase miRNA silencing activity (Wang *et al.* 2006). Further studies indicated that miRISC-associated mRNAs are present in cytoplasmic foci called P-bodies, which are known sites of mRNA destabilization or storage and release (Peters and Meister 2007), suggesting that general mRNA turnover pathways might play a role in miRNA-mediated mRNA decay (Liu *et al.* 2005; Meister *et al.* 2005; Rehwinkel *et al.* 2005; Valencia-Sanchez *et al.* 2006). Indeed, it has been reported that miRNA-mediated silencing might have a profound impact on target mRNA levels, most likely by directing deadenylation and decapping of the mRNA (Bagga *et al.* 2005; Giraldez *et al.* 2006; Lim *et al.* 2005; Valencia-Sanchez *et al.* 2006; Wu *et al.* 2006). In addition, Ago proteins were also found to localize to the diffuse cytoplasm and stress granules (SGs) under stresses such as heat shock or oxidative stress, implicating another type of miRNA-mediated gene regulation (Leung *et al.* 2006). The physiological role of miRISC-associated mRNA accumulation in SGs during stress response remains unclear.

2.22.2.5 miRNA in the Regulation of Cellular Processes

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p0085 Increasing evidence indicates that animal miRNAs play a pivotal role in a variety of cellular processes, including cell proliferation, cell differentiation, developmental timing, fat metabolism, apoptosis, insulin secretion, stem cell maintenance, neuronal patterning, and hematopoietic lineage differentiation (**Table 1**). The function of unique miRNAs in animals has been analyzed either genetically or by delivery of synthetic pre-miRNAs to knock in or antagomirs to knock down (Krutzfeldt *et al.* 2006). Many computational methods have also been developed to define miRNA regulatory networks (Rajewsky 2006); yet most molecular targets of miRNAs remain experimentally undefined. Normally, a single miRNA is predicted to repress and destabilize 100–200 target mRNAs (Krutzfeldt

t0005 **Table 1** miRNAs in the regulation of cellular process

Biological function	*miRNA*	*Targets*	*References*
Cardiomyocyte/skeletal muscle differentiation and proliferation	miR-1	*Hand2*, *Hdac4*, *Gja1*, and *Kcnj2*	Chen *et al*. (2006), Nakajima *et al*. (2006), Yang *et al*. (2007), Zhao *et al*. (2005, 2007)
	miR-133	*Nptb* and *Srf*	Boutz *et al*. (2007), Chen *et al*. (2006)
	miR-181	*Hox-A11*	Naguibneva *et al*. (2006)
	miR-206	Id1-3, MyoR, *Fstl1*, and *Utrn*	Kim *et al*. (2006), Rosenberg *et al*. (2006)
	miR-214	*su(fu)*	Flynt *et al*. (2007)
Apoptosis	bantam	*Hid*	Brennecke *et al*. (2003)
	miR-14	*Drice, Dep-1, Scythe, SkpA, and Grim*	Xu *et al*. (2003)
	miR-15a/miR-16-1	*BCL2*	Cimmino *et al*. (2005)
	miR-21	*Tpm1*, *Pten*, and *Pdcd4*	Asangani *et al*. (2007), Frankel *et al*. (2007), Si *et al*. (2007)
	miR-29b	*Mcl-1*	Mott *et al*. (2007)
	miR-34a	*E2F3*	Welch *et al*. (2007)
	miR-184	N/A	Welch *et al*. (2007)
Regulator of cholesterol and fatty acid metabolism	miR-14	*EcR*	Varghese and Cohen (2007)
	miR-103/miR-107	Multiple genes involving cellular acetyl-CoA and lipid levels	Wilfred *et al*. (2007)
	miR-122	*Ho-1* and *Slc7a1* (*Cat-1*)	Bhattacharyya *et al*. (2006), Chang *et al*. (2004), Esau *et al*. (2006), Shan *et al*. (2007)
	miR-143	*Erk5*	Esau *et al*. (2006)
	miR-278	*Expanded*	Teleman *et al*. (2006)
Regulation of pancreatic development and insulin secretion	miR-9	*Granuphilin/Slp4*	Plaisance *et al*. (2006)
	miR-15a, miR-15b, miR-16, miR-195	*Ngn3*	Joglekar *et al*. (2007a,b)
	miR-124a	*Foxa2*	Baroukh *et al*. (2007)
	miR-214, miR-503, miR-541	N/A	Joglekar *et al*. (2007a,b)
	miR-375	*Mtpn*	Kloosterman *et al*. (2007), Poy *et al*. (2004)
Hematopoietic differentiation	miR-10a	*Hoxa1*	Garzon *et al*. (2006)
	miR-16	N/A	Bruchova *et al*. (2007)
	miR-30a	*Mafb*	Garzon *et al*. (2006)
	miR-142-5p	N/A	Chen *et al*. (2004)
	miR-150	*c-Myb*	Xiao *et al*. (2007), Zhou *et al*. (2007)
	miR-155	*C/EBβ*, *Crebbp*, *Jun*, *Meis1*, *Pu.1*, *Agtr1*, *Agtr2*, and *Fos*	Georgantas *et al*. (2007), Masaki *et al*. (2007)
	miR-181	N/A	Chen *et al*. (2004)
	miR-221/miR-222	*c-Kit*	Felli *et al*. (2005)
	miR-223	*Nf1a*	Fazi *et al*. (2005)
	miR-451	N/A	Bruchova *et al*. (2007), Masaki *et al*. (2007)
Neuronal morphogenesis	miR-9, miR-23, miR-26, miR-29, miR-125, miR-128	N/A	Smirnova *et al*. (2005)

	miR-23b	*Hes1*[a]	Kimura *et al*. (2004)
	miR-124a	Probably many nonneuronal transcripts	Smirnova *et al*. (2005), Conaco *et al*. (2006)
	miR-132	*Creb*	Vo *et al*. (2005)
	miR-134	*Limk1*	Schratt *et al*. (2006)
	miR-430	Probably many maternal mRNAs	Giraldez *et al*. (2006)
Stem cell maintenance, regeneration, and homeostasis	miR-290, miR-291, miR-292, miR-293, miR-294, miR-295miR-302b/302b*, miR-302c/302c*, miR-302a/302a*, miR-302d, miR-367, miR-371, miR-372, miR-373/373*	*Notch*, *Ids*, and *Pten*	Cheng *et al*. (2005), Houbaviy *et al*. (2003), Suh *et al*. (2004)
Cell cycle regulation	miR-16-1	*Cdk6*	Linsley *et al*. (2007), Raveche *et al*. (2007)
	miR-17-5p	*E2F1*	O'Donnell *et al*. (2005)
	miR-29b	N/A	Hwang *et al*. (2007)
	miR-34	*Cdk4* and *Met*	He *et al*. (2007b)
	miR-127	*Bcl6*	Saito *et al*. (2006)
	miR-184	N/A	Welch *et al*. (2007)
	miR-221/miR-222	*c-Kit*	Felli *et al*. (2005)
Skin morphogenesis and hair follicles development	miR-17-5p(a), miR-19b, miR-20, miR-93, miR-141, miR-199a-b, miR-200a-c, miR-429	N/A	Andl *et al*. (2006), Yi *et al*. (2006)
Angiogenesis	let-7 family	*Tsp1*	Kuehbacher *et al*. (2007)
	miR-17-92	*Tsp1*	Dews *et al*. (2006)
	miR-27b	Antiangiogenic genes	Kuehbacher *et al*. (2007)
	miR-155	*At1r*	Martin *et al*. (2006)
	miR-221/miR-222	*c-Kit*	Poliseno *et al*. (2006)
Immune response	miR-132		Taganov *et al*. (2006)
	miR-146	*Traf6* and *Irak1*	Taganov *et al*. (2006)
	miR-155	*Pu.1*	O'Connell *et al*. (2007), Rodriguez *et al*. (2007), Taganov *et al*. (2006)
Circadian clock regulation	miR-132	*Rfx4*	Cheng *et al*. (2007)
	miR-219	*Scop*	Cheng *et al*. (2007)
Developmental patterning	miR-15/miR-16	*Acvr2a*	Martello *et al*. (2007)
	miR-196a	*Hoxb8*	Hornstein *et al*. (2005)
Antiviral defense	miR-1, miR-30, miR-128, miR-196, miR-296, miR-351, miR-431, miR-448	Hepatitis C virus	Pedersen *et al*. (2007)
	miR-32	Retrovirus *Pfv-1*	Lecellier *et al*. (2005)
	miR-29a/miR-29b, miR-149, miR-378, miR-324-5p	*Nef*, *Vpr*, *Env*, and *Vif*	Hariharan *et al*. (2005)
	Predicted host miRNAs	Virus genes	Hsu *et al*. (2007)
Replication of hepatitis C virus	miR-122	Hepatitis C virus	Jopling *et al*. (2005)

[a] This target is problematic as the authors have retracted the paper since then.
N/A, not available.

et al. 2005; Legendre *et al.* 2006; Lim *et al.* 2005; Linsley *et al.* 2007). **Table 1** summarizes the miRNAs that are associated with biological processes and some of the target genes through which they exert their regulatory function in animals.

p0090 Since Dicer is an essential enzyme in miRNA biogenesis (Jiang *et al.* 2005; Lee *et al.* 2004b; Saito *et al.* 2005), a good approach to study the global role of miRNAs in animal development is to use *dicer* mutants. Evidence in various organisms indicates that loss of function of *dicer* results in animal developmental abnormalities and death. For example, loss of function of *dicer* in zebrafish results in defects during gastrulation and brain morphogenesis (Giraldez *et al.* 2005) and in developmental arrest around day 10 (Wienholds *et al.* 2003). Loss of function of *dicer* in mice led to lethality at embryonic day 7.5, with embryos being small, appearing morphologically abnormal and devoid of pluripotent stem cells (Bernstein *et al.* 2003). As expected, Dicer-deficient mouse embryonic stem (ES) cells are defective in differentiation both *in vitro* and *in vivo* and do not form the three germ layers normally found in embryoid bodies derived from ES cells (Kanellopoulou *et al.* 2005). Furthermore, conditional inactivation of Dicer to circumvent the embryonic lethality of Dicer-null mutants suggested that miRNAs are essential for morphogenesis of the vertebrate limb (Harfe *et al.* 2005), skin (Andl *et al.* 2006; Yi *et al.* 2006), and respiratory epithelium (Harris *et al.* 2006). In addition, Dicer deficiency revealed that miRNAs might play an integral role in germline maintenance and organization and control of postmeiotic male germ cell differentiation in many organisms (Hatfield *et al.* 2005; Jin and Xie 2007; Kotaja *et al.* 2006). Recent studies indicate that mouse oocytes lacking Dicer arrest in meiosis I with multiple disorganized spindles and severe chromosome congression defects, suggesting that miRNAs are indispensable for mouse oogenesis (Murchison *et al.* 2007; Tang *et al.* 2007).

p0095 Likewise, depletion in mouse ES cells of DGCR8, essential for Drosha processing of pri-miRNA, severely impaired miRNA biogenesis and the resulting embryos were arrested early in development. However, unlike Dicer1 knockout ES cells, DGCR8-deficient ES cells do retain pluripotency under induction and continue to grow and differentiate even after 16 days (Wang *et al.* 2007). This different phenotype might be explained by miRNA-independent functions of Dicer, such as siRNA production (Bernstein *et al.* 2001; Ketting *et al.* 2001), and/or the Drosha/DGCR8-independent mirtronic miRNA functioning (Okamura *et al.* 2007; Ruby *et al.* 2007).

2.22.3 Pathological Roles of Human miRNAs

s0040

p0100 Aberrant expression of miRNA appears to be a pathological feature of numerous diseases, including cancer, cardiovascular diseases, viral infection, metabolic disorders, and innate immune response disorders. The majority of human miRNA genes are located at fragile sites and genomic regions involved in cancers (Calin *et al.* 2004), and dysfunctional expression of miRNA is frequently observed in many human malignancies. Accumulating data suggest that miRNAs play pivotal roles in tumorigenesis, classification, diagnosis, treatment response, and prognosis predication. Additionally, several studies have shown that miRNAs are involved in the pathogenesis of cardiovascular disorders. miRNA represents a vital component of the innate antiviral immune response in plants, invertebrates, and mammals. Evidence shows that numerous viruses interact with the miRNA machinery and that a number of viruses encode their own miRNAs. Virus-encoded miRNAs seem to evolve rapidly and regulate both the viral life cycle and the interaction with their hosts. Recently, miRNAs have been found to be directly involved in the fine-tuning of innate immunity responses and transduction signaling by toll-like receptors and the ensuing cytokine response. Therefore, mimicking or inhibiting miRNA activity could have an impact on disease progression and suggests that miRNAs have potential as therapeutics.

2.22.3.1 miRNAs in Cancer

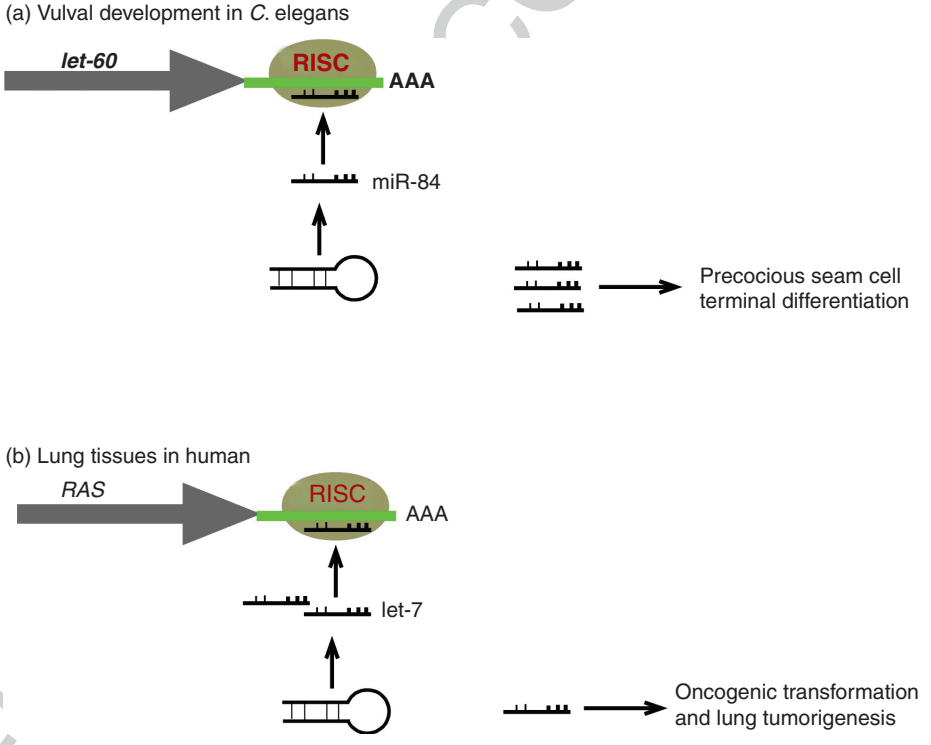
s0045

p0105 Studies of miRNA expression profiles in cancer samples have identified a group of miRNAs that are differentially regulated in tumors, suggesting a possible link between miRNAs and tumorigenesis (Lim *et al.* 2005). Croce and colleagues first identified two miRNA genes, *mir-15a* and *mir-16-1*, that are located on chromosome 13q14, a region deleted in more than half of chronic lymphocytic leukemia (CLL) patients. They demonstrated that the expression of *mir-15a* and *mir-16-1* is inversely correlated with the expression of *BCL2*. The *BCL2* gene produces B-cell lymphoma 2 protein, which is antiapoptotic. It was found that these two miRNAs induce apoptosis by repressing *BCL2* in a leukemia cell line model, suggesting that miRNAs may contribute to CLL development (Cimmino *et al.* 2005).

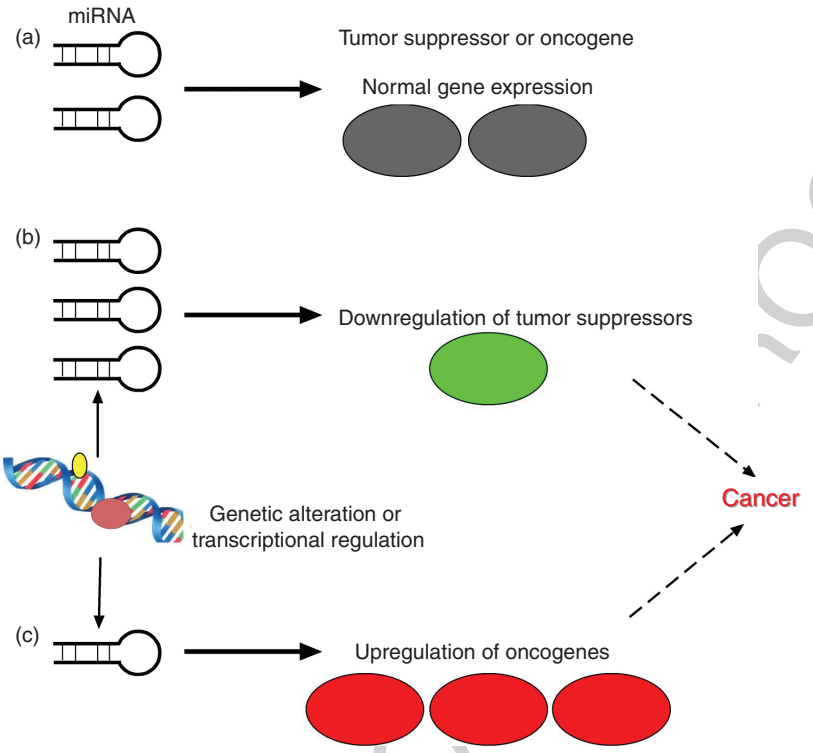
p0110 The studies of the molecular oncology of lung cancer have traditionally concentrated on protein-coding genes such as *RAS*, *p53*, and *Rb* (Meuwissen *et al.* 2003). It is only recently that investigations have revealed a correlation between miRNA and lung cancer. First, a group of Japanese scientists found that let-7 miRNA (a homolog of the *C. elegans* let-7 miRNA) was downregulated in human lung cancer and the reduced expression of *let-7* is associated with shortened postoperative survival (Takamizawa *et al.* 2004). Frank Slack (one of the authors who discovered the let-7 miRNA in *C. elegans*) and colleagues reported that the *C. elegans* miRNA miR-84 negatively regulates the *RAS* gene (called *let-60* in *C. elegans*) (**Figure 3a**). miRNA miR-84 is a member of the *C. elegans* let-7 miRNA family consisting of miR-48, miR-84, miR-241, and let-7 (Abbott *et al.* 2005). This family displays high sequence identity, with particular conservation at the 5′ end of the mature miRNAs. Overexpression of *mir-84* in *C. elegans* suppresses *let-60*/*RAS* gene function and induces precocious seam cell terminal differentiation (Abbott *et al.* 2005).

p0115 The human *RAS* gene is a well-defined oncogene that is commonly mutated in lung and other cancers (Malumbres and Barbacid 2003). Overexpression of *RAS* results in oncogenic transformation of human cells (McKay *et al.* 1986; Pulciani *et al.* 1985). The Slack laboratory showed that significantly higher levels of RAS proteins correlated with lower *let-7* gene expression in human lung tumors. Moreover, let-7 miRNA downregulated *RAS* genes (*KRAS*, *HRAS*, and *NRAS*) by partially complementing their 3′-UTRs in a cultured cell model (Johnson *et al.* 2005) (**Figure 3b**). These results emphasize the importance of miRNA in the pathogenesis of lung cancer and the potential to target miRNA expression as an effective approach for therapeutic intervention.

p0120 The pioneering work on let-7 and *RAS*, and studies on other cancer-related miRNAs (Ambion 2005; Chan *et al.* 2005; Cimmino *et al.* 2005) have led to a



f0015 **Figure 3** miRNA-mediated regulation of RAS expression in *Caenorhabditis elegans* and human. In specific vulval precursor cells from worms (a) and in normal human lung tissue (b), *mir-84*, a member of the *let-7* miRNA family, and *let-7* are transcribed, respectively, and the transcripts, which have characteristic hairpin structures, are processed into mature miRNAs. These transcripts are then incorporated into RISC. miRNA species guide miRNA-associated RISC to target mRNAs by hybridizing to complementary sequences in the 3′-UTRs of mRNAs and thereby prevent their translation. Slack and colleagues showed that members of the let-7 family repress the expression of *RAS* genes and that this mechanism is potentially relevant to the pathogenesis of lung cancer.



f0020 **Figure 4** A model of miRNA involvement in cancer by modulation of expression of tumor suppressor genes or oncogenes. (a) miRNA regulates its target gene expression. (b) Overexpression of miRNAs, for instance, by amplification of the miRNA-encoding locus, could decrease expression of a target, such as a tumor suppressor gene. (c) Underexpression of miRNAs, for instance, by deletion or methylation of an miRNA locus, could result in increased expression of a target such as an oncogene.

suggested framework for understanding the role of miRNAs in cancer: miRNA-mediated tumorigenesis results from either downregulation of tumor suppressor or upregulation of oncogenes (**Figure 4**). The relationship between *let*-7 and *RAS* is a paradigm of the suppressor (*let*-7) and the oncogene (*RAS*). The opposite scenario is also equally intriguing: an miRNA acts as an oncogene to repress gene expression of a tumor suppressor. miRNA dysregulation in various cancers is summarized in **Table 2**.

s0050 ### 2.22.3.2 miRNAs in Cardiovascular Diseases

p0125 Studies of miRNAs in cardiovascular biology and disease have been limited compared to that in cancer. Nevertheless, there have been some groundbreaking results since 2005. miR-1 was found to be specifically expressed in cardiac and skeletal muscle precursor cells and the *mir-1* gene was a direct transcriptional target of muscle differentiation regulators including SRFs (Zhao *et al.* 2005). Excess miR-1 in the developing heart leads to a decreased pool of proliferating ventricular cardiomyocytes, suggesting that miR-1 modulates the effects of critical cardiac regulatory proteins to control the balance between differentiation and proliferation during cardiogenesis (Zhao *et al.* 2005). The role of miR-1 in the regulation of cardiac morphogenesis, electrical conduction, and cell cycle control was further confirmed in a mouse knockout model (Zhao and Srivastava 2007). Ensuing articles reported that another miRNA, miR-133, which is in the same polycistron as miR-1, enhances myoblast proliferation by repressing SRF (Chen *et al.* 2006) and controls cardiomyocytic hypertrophy (Care *et al.* 2007). A large number of miRNAs (113 out of 140 that are expressed in normal rat carotid arteries) are aberrantly expressed on rat vascular walls within the neointimal lesion using microarray analysis in a balloon injury rat model. This phenotype is believed to be a common and early pathological feature of atherosclerosis (Ji *et al.* 2007). Downregulation of miR-21, the most upregulated miRNA in balloon-injured rat carotid arteries, inhibited neointimal lesion formation after angioplasty (Ji *et al.* 2007). Readers interested in miRNA biology

t0010 **Table 2** miRNAs in human cancer

Classification/ morphology	*Tumor type*	*miRNAs dysregulated*	*References*
Epithelial cells	Eight types of solid organ tumors	miR-21 is upregulated in tumors of lung, breast, stomach, prostate, colon, head and neck, esophagus, and pancreas	Chan *et al*. (2005), Diederichs and Haber (2006), Iorio *et al*. (2005), Roldo *et al*. (2006), Volinia *et al*. (2006)
	Lung bronchial adenocarcinoma	Reduced let-7 expression; upregulated miR-155; overexpression of miR-17–92 cluster	Hayashita *et al*. (2005), Johnson *et al*. (2005), Volinia *et al*. (2006)
	Breast adenocarcinoma	Overexpression of miR-155; downregulation of miR-10b, miR-125b, and miR-145	Iorio *et al*. (2005)
	Colorectal adenocarcinoma	Downregulation of miR-143 and miR-145; miR-34a was highly upregulated in a human colon cancer cell line	Akao *et al*. (2007a,b), Tazawa *et al*. (2007)
	Prostate adenocarcinoma	Downregulation of miR-125b, miR-145, and let-7c; overexpression of miR-221 and miR-222; loss of miR-146a function	Galardi *et al*. (2007), Ozen *et al*. (2007), Lin *et al*. (2008)
	Head and neck squamous cell carcinoma	Upregulation of miR-205	Tran *et al*. (2007)
	Esophageal squamous cell carcinoma	miR-203 and miR-205 upregulation	Feber *et al*. (2008)
	Cervical carcinoma	Reduced expression of miR-143; increased expression of miR-21	Lui *et al*. (2007)
	Hepatocellular carcinoma	Upregulation of miR-98 and miR-148a; downregulation of miR-198	Huang *et al*. (2008)
	Gastric adenocarcinoma	Reduced let-7a expression	Zhang *et al*. (2007)
	Thyroid papillary carcinoma	Upregulation of miR-221, miR-222, and miR-146	He *et al*. (2005a), Visone *et al*. (2007)
	Pancreatic ductal adenocarcinoma (PDAC)	miR-155, which is overexpressed in PDAC cells; miR-34a, frequently absent in pancreatic cancer cells; miR-221, miR-376a, and miR-301 are overexpressed; the expression of miR-103 and miR-107, associated with lack of expression of miR-155, discriminates tumors from normal.	Chang *et al*. (2007), Gironella *et al*. (2007), Lee *et al*. (2007), Roldo *et al*. (2006)
Connective tissues	Sarcomas	miR-143 was highly expressed in the majority of leiomyosarcomas (LMSs) and all gastrointestinal stromal tumors (GISTs); other dysregulated miRNAs	Subramanian *et al*. (2007)

(*Continued*)

Table 2 (Continued)

Classification/ morphology	*Tumor type*	*miRNAs dysregulated*	*References*
Hematopoietic cells	Acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML)	miR-128a and miR-128b are significantly overexpressed in ALL compared with AML, whereas let-7b and miR-223 are downregulated	Mi *et al*. (2007)
	Precursor B-cell ALL	Insertion of miRNA-125b-1 into a rearranged immunoglobulin heavy chain gene locus in a patient with precursor B-cell ALL	Sonoki *et al*. (2005)
	Chronic lymphocytic leukemia (CLL)	miR-15a and miR-16-1 downregulated; miR-155/BIC upregulated	Cimmino *et al*. (2005), Fulci *et al*. (2007)
	Adult T-cell leukemia	miR-105–363 cluster overexpression	Landais *et al*. (2007)
	Acute erythroleukemia	miR-17–92 cluster overexpression	Cui *et al*. (2007)
	Mantle cell lymphoma	miR-17–92 cluster amplification	Rinaldi *et al*. (2007)
	Burkitt lymphoma	Lack of miR-155 in the BIC transcript	Kluiver *et al*. (2006)
Nervous system (neuronal and stromal cells)	Glioblastoma	miRNA-21 is an antiapoptotic factor in human glioblastoma cells; miRNA-21 knockdown disrupts glioma growth *in vivo*; miR-221, strongly upregulated in glioblastoma and from a set of brain-enriched miRNAs, miR-128, miR-181a, miR-181b, and miR-181c, which are downregulated in glioblastoma.	Chan *et al*. (2005), Ciafre *et al*. (2005), Corsten *et al*. (2007)
	Neuroblastoma	Downregulation of miR-34a	Welch *et al*. (2007)
Stem cell or germ cell	Germ cell tumor	Overexpression of miR-372 and miR-373	Gillis *et al*. (2007), Voorhoeve *et al*. (2007)

in cardiovascular diseases are referred to a comprehensive review by Latronico *et al.* (2007) published by *Circulation Research.*

s0055 ## 2.22.4 miRNAs in Toxicology

p0130 Though many miRNA studies in health and disease are relevant to toxicology, there are few reports that directly address miRNA expression or function in molecular toxicology. A National Center for Biotechnology Information (NCBI) PubMed search of 'microRNA' and 'toxicology' returns only one original research paper, in which Horii and colleagues (Pfizer Japan, Inc.) have investigated miRNA expression and roles of gene regulation in rat livers treated with two hepatotoxicants (Fukushima *et al.* 2007). These investigators exposed male rats to a single dose of acetaminophen (the active ingredient of the blockbuster drug Tylenol) or carbon tetrachloride via gavage and rat livers were then removed for miRNA expression profiling. These studies found that six miRNAs were upregulated by both toxicants, while eight were downregulated. miR-298 and miR-370 (both of which were downregulated) were chosen for further analyses as these two miRNAs were predicted to target a gene coding for thioredoxin reductase 3, an enzyme responsive to oxidative stress (Sun *et al.* 1999). It was found that miR-298 and miR-370 were suppressed around the time when mitochondria were severed prior to cell collapse, indicating that miRNA dysregulation occurs at the early stages of toxicity. The mechanism of miRNA dysregulation under toxicant treatment needs further investigation; yet this report suggests the importance of miRNA in toxicity.

p0135 A large number of miRNAs listed in **Tables 1** and **2** should be considered as potential players in cellular response to toxin insults. For example, miR-34a, miR-34b, and miR-34c are regulated by p53 and they would be expected to be induced should p53 become activated. He *et al.* (2007a) reported that upon DNA damage by adriamycin treatment, a large number of miRNAs are dysregulated in epithelial ovarian cancer TOV21G cells with miR-34a, miR-34b, and miR-34c being among the highest upregulated. This result suggests that when cells are subjected to genotoxic agents, miR-34a, miR-34b, and miR-34c may be induced by activated p53 protein.

s0060 ## 2.22.5 Perspectives on miRNA-Based Therapeutics

p0140 Rapid advances have revealed the physiological and pathological functions of miRNA, while miRNA dysregulation has been found in many diseases and pathological states, particularly in neoplastic and cardiovascular diseases. Though miRNAs represent a class of regulatory genes with great potential for use in diagnosis, prognosis, and therapy, further studies are needed to advance the basic research of miRNA biology and to apply the knowledge of scientific discoveries to clinical diagnosis, classification, prediction for prognosis, and treatment response. Particularly, there are several critical questions that need to be answered before we ponder the use of miRNA in therapeutic intervention.

s0065 ### 2.22.5.1 Multiple Targets

p0145 An miRNA normally consists of an average of ~22 nt. The seed sequence (position 2–8) is the most important region for target recognition. Given that the last nucleotide can be mutated, the specificity of these seed nucleotides is about $1/4^6 = 1/4096$ (without considering the G:U or U:G wobble base pairs), that is for *any* UTR that is over 4 kb, there will be a target site for *any* miRNA. Other general features of site context that boost site efficacy such as AU-rich nucleotide composition near the site, proximity to sites for coexpressed miRNAs (which leads to cooperative action), proximity to residues pairing to miRNA nucleotides 13–16, positioning within the 3′-UTR at least 15 nt from the stop codon, and positioning away from the center of long UTRs have been reported recently (Grimson *et al.* 2007). However, even these seed matches are not always sufficient for repression, and the above-mentioned features are unlikely to improve the specificity of miRNA targeting to another 1/1000. Current computational predictions indicate that each miRNA targets hundreds if not thousands of genes (Grimson *et al.* 2007; Krek *et al.* 2005; Miranda *et al.* 2006). Experimentally, however, most targets have not been validated.

s0070 ### 2.22.5.2 Multiple miRNA Genes

p0150 The let-7 genes in human include let-7a to let-7i (eight miRNAs), of which let-7a has three copies and let-7f has two copies (11 in total). The mouse let-7 genes include the same eight miRNAs, of which

let-7a, let-7c, and let-7f each have two copies (11 in total). Knocking out any let-7 gene or gene cluster in the mouse did not generate any tangible phenotype. Interestingly, most publications on let-7 have not explicitly identified which let-7 is dysregulated beyond let-7a. Also, mammalian let-7s have the same seed sequence as miR-98, further complicating the study of this gene family.

s0075 2.22.5.3 miRNA Functions *In Vivo*

p0155 miRNAs, siRNAs, or antisense oligonucleotides that target miRNA are RNA molecules that may or may not be modified. RNA-based drug development has not been highly successful and only one drug has been approved by the Food and Drug Administration (FDA) for use in clinics (Vitravene, i.e., fomivirsen, is the first and only antisense drug to achieve marketing clearance; Vitravene treats a condition called cytomegalovirus (CMV) retinitis in people with acquired immunodeficiency syndrome (AIDS); Isis Pharmaceuticals (Carlsbad, CA, USA) developed the drug and licensed the worldwide commercial rights to Novartis Ophthalmics). Small molecules work by binding to a target protein and prevent it from functioning, ensure it functions better, or allow it to function at different times. The promise of antisense or RNA drugs is that they might prevent proteins from ever being produced. However, the complexities of the inhibitory mechanism(s) and off-target effects *in vivo* have not been fully appreciated. In addition, it remains unclear how many genes are targeted by a given miRNA or antisense.

s9000 2.22.5.4 Conclusions

p0160 To summarize, in this chapter we have focused on recent discoveries that elucidate miRNA biological functions as well as their crucial roles in human diseases, particularly in neoplastic and cardiovascular diseases. miRNA-based therapy is anticipated to become a new technology of choice for treating a wide range of human diseases. We are cautiously optimistic about miRNA-based therapeutics. As most miRNA genes are transcribed by RNA polymerase II and controlled by transcription factors (such as miR-34a/b/c by p53), they are undoubtedly important players and harbor significant potential for applications in molecular and cellular toxicology.

Acknowledgments

Yong Li is supported by the Career Development Program and a pilot grant from the Center for Genomics and Integrated Biology at University of Louisville funded by NIEHS P30ES014443. Liu M-F is supported by grants from the Ministry of Science and Technology of China (2005CB724603) and the National Natural Science Foundation of China (30770474, 90919016, and 30970621).

References

Abbott, A. L.; Alvarez-Saavedra, E.; Miska, E. A.; Lau, N. C.; Bartel, D. P.; Horvitz, H. R.; Ambros, V. *Dev. Cell* **2005**, *9*(3), 403–414. b0005

Akao, Y.; Nakagawa, Y.; Kitade, Y.; Kinoshita, T.; Naoe, T. *Cancer Sci.* **2007a**, *98*(12), 1914–1920. b0010

Akao, Y.; Nakagawa, Y.; Naoe, T. *DNA Cell Biol.* **2007b**, *26*(5), 311–320. b0015

Altuvia, Y.; Landgraf, P.; Lithwick, G.; Elefant, N.; Pfeffer, S.; Aravin, A.; Brownstein, M. J.; Tuschl, T.; Margalit, H. *Nucleic Acids Res.* **2005**, *33*(8), 2697–2706. b0020

Ambion. *Ambion Tech. Notes* **2005**, *12*(1), 1. b0025

Andl, T.; Murchison, E. P.; Liu, F.; Zhang, Y.; Yunta-Gonzalez, M.; Tobias, J. W.; Andl, C. D.; Seykora, J. T.; Hannon, G. J.; Millar, S. E. *Curr. Biol.* **2006**, *16*(10), 1041–1049. b0030

Asangani, I. A.; Rasheed, S. A.; Nikolova, D. A.; Leupold, J. H.; Colburn, N. H.; Post, S.; Allgayer, H. *Oncogene* **2007**, *27*, 2128–2136. b0035

Bagga, S.; Bracht, J.; Hunter, S.; Massirer, K.; Holtz, J.; Eachus, R.; Pasquinelli, A. E. *Cell* **2005**, *122*(4), 553–563. b0040

Baroukh, N.; Ravier, M. A.; Loder, M. K.; Hill, E. V.; Bounacer, A.; Scharfmann, R.; Rutter, G. A.; Van Obberghen, E. *J. Biol. Chem.* **2007**, *282*(27), 19575–19588. b0045

Bartel, D. P. *Cell* **2004**, *116*(2), 281–297. b0050

Baskerville, S.; Bartel, D. P. *RNA* **2005**, *11*(3), 241–247. b0055

Berezikov, E.; Guryev, V.; van de Belt, J.; Wienholds, E.; Plasterk, R. H.; Cuppen, E. *Cell* **2005**, *120*(1), 21–24. b0060

Bernstein, E.; Caudy, A. A.; Hammond, S. M.; Hannon, G. J. *Nature* **2001**, *409*(6818), 363–366. b0065

Bernstein, E.; Kim, S. Y.; Carmell, M. A.; Murchison, E. P.; Alcorn, H.; Li, M. Z.; Mills, A. A.; Elledge, S. J.; Anderson, K. V.; Hannon, G. J. *Nat. Genet.* **2003**, *35*(3), 215–217. b0070

Bhattacharyya, S. N.; Habermacher, R.; Martine, U.; Closs, E. I.; Filipowicz, W. *Cell* **2006**, *125*(6), 1111–1124. b0075

Blencowe, B. J.; Bowman, J. A.; McCracken, S.; Rosonina, E. *Biochem. Cell Biol.* **1999**, *77*(4), 277–291. b0080

Boggs, R. M.; Moody, J. A.; Long, C. R.; Tsai, K. L.; Murphy, K. E. *Gene* **2007**, *404*(1–2), 25–30. b0085

Borchert, G. M.; Lanier, W.; Davidson, B. L. *Nat. Struct. Mol. Biol.* **2006**, *13*(12), 1097–1101. b0090

Boutz, P. L.; Chawla, G.; Stoilov, P.; Black, D. L. *Genes Dev.* **2007**, *21*(1), 71–84. b0095

Brennecke, J.; Hipfner, D. R.; Stark, A.; Russell, R. B.; Cohen, S. M. *Cell* **2003**, *113*(1), 25–36. b0100

Bruchova, H.; Yoon, D.; Agarwal, A. M.; Mendell, J.; Prchal, J. T. *Exp. Hematol.* **2007**, *35*(11), 1657–1667. b0105

Calin, G. A.; Sevignani, C.; Dumitru, C. D.; Hyslop, T.; Noch, E.; Yendamuri, S.; Shimizu, M.; Rattan, S.; Bullrich, F.; Negrini, M., *et al. Proc. Natl. Acad. Sci. USA* **2004**, *101*(9), 2999–3004. b0110

Care, A.; Catalucci, D.; Felicetti, F.; Bonci, D.; Addario, A.; Gallo, P.; Bang, M. L.; Segnalini, P.; Gu, Y.; Dalton, N. D., *et al. Nat. Med.* **2007**, *13*(5), 613–618. b0115

b0120 Carmell, M. A.; Hannon, G. J. *Nat. Struct. Mol. Biol.* **2004**, *11*(3), 214–218.
b0125 Cerutti, L.; Mian, N.; Bateman, A. *Trends Biochem. Sci.* **2000**, *25*(10), 481–482.
b0130 Chan, J. A.; Krichevsky, A. M.; Kosik, K. S. *Cancer Res.* **2005a**, *65*(14), 6029–6033.
b0135 Chang, J.; Nicolas, E.; Marks, D.; Sander, C.; Lerro, A.; Buendia, M. A.; Xu, C.; Mason, W. S.; Moloshok, T.; Bort, R., *et al. RNA Biol.* **2004**, *1*(2), 106–113.
b0140 Chang, T. C.; Wentzel, E. A.; Kent, O. A.; Ramachandran, K.; Mullendore, M.; Lee, K. H.; Feldmann, G.; Yamakuchi, M.; Ferlito, M.; Lowenstein, C. J., *et al. Mol. Cell* **2007**, *26*(5), 745–752.
b0145 Chen, C. Z.; Li, L.; Lodish, H. F.; Bartel, D. P. *Science* **2004**, *303*(5654), 83–86.
b0150 Chen, J. F.; Mandel, E. M.; Thomson, J. M.; Wu, Q.; Callis, T. E.; Hammond, S. M.; Conlon, F. L.; Wang, D. Z. *Nat. Genet.* **2006**, *38*(2), 228–233.
b0155 Chendrimada, T. P.; Gregory, R. I.; Kumaraswamy, E.; Norman, J.; Cooch, N.; Nishikura, K.; Shiekhattar, R. *Nature* **2005**, *436*(7051), 740–744.
b0160 Cheng, H. Y.; Papp, J. W.; Varlamova, O.; Dziema, H.; Russell, B.; Curfman, J. P.; Nakazawa, T.; Shimizu, K.; Okamura, H.; Impey, S., *et al. Neuron* **2007**, *54*(5), 813–829.
b0165 Cheng, L. C.; Tavazoie, M.; Doetsch, F. *Neuron* **2005**, *46*(3), 363–367.
b0170 Ciafre, S. A.; Galardi, S.; Mangiola, A.; Ferracin, M.; Liu, C. G.; Sabatino, G.; Negrini, M.; Maira, G.; Croce, C. M.; Farace, M. G. *Biochem. Biophys. Res. Commun.* **2005**, *334*(4), 1351–1358.
b0175 Cimmino, A.; Calin, G. A.; Fabbri, M.; Iorio, M. V.; Ferracin, M.; Shimizu, M.; Wojcik, S. E.; Aqeilan, R. I.; Zupo, S.; Dono, M., *et al. Proc. Natl. Acad. Sci. USA* **2005**, *102*(39), 13944–13949.
b0180 Conaco, C.; Otto, S.; Han, J. J.; Mandel, G. *Proc. Natl. Acad. Sci. USA* **2006**, *103*(7), 2422–2427.
b0185 Corsten, M. F.; Miranda, R.; Kasmieh, R.; Krichevsky, A. M.; Weissleder, R.; Shah, K. *Cancer Res.* **2007**, *67*(19), 8994–9000.
b0190 Cui, J. W.; Li, Y. J.; Sarkar, A.; Brown, J.; Tan, Y. H.; Premyslova, M.; Michaud, C.; Iscove, N.; Wang, G. J.; Ben-David, Y. *Blood* **2007**, *110*(7), 2631–2640.
b0195 Cullen, B. R. *Mol. Cell* **2004**, *16*(6), 861–865.
b0200 Denli, A. M.; Tops, B. B.; Plasterk, R. H.; Ketting, R. F.; Hannon, G. J. *Nature* **2004**, *432*(7014), 231–235.
b0205 Dews, M.; Homayouni, A.; Yu, D.; Murphy, D.; Sevignani, C.; Wentzel, E.; Furth, E. E.; Lee, W. M.; Enders, G. H.; Mendell, J. T., *et al. Nat. Genet.* **2006**, *38*(9), 1060–1065.
b0210 Diederichs, S.; Haber, D. A. *Cancer Res.* **2006**, *66*(12), 6097–6104.
b0215 Diederichs, S.; Haber, D. A. *Cell* **2007**, *131*(6), 1097–1108.
b0220 Doench, J. G.; Sharp, P. A. *Genes Dev.* **2004**, *18*(5), 504–511.
b0225 Esau, C.; Davis, S.; Murray, S. F.; Yu, X. X.; Pandey, S. K.; Pear, M.; Watts, L.; Booten, S. L.; Graham, M.; McKay, R., *et al. Cell Metab.* **2006**, *3*(2), 87–98.
b0230 Esquela-Kerscher, A.; Slack, F. J. *Nat. Rev. Cancer* **2006**, *6*(4), 259–269.
b0235 Fazi, F.; Rosa, A.; Fatica, A.; Gelmetti, V.; De Marchis, M. L.; Nervi, C.; Bozzoni, I. *Cell* **2005**, *123*(5), 819–831.
b9000 Feber, A.; Xi, L.; Luketich, J. D.; Pennathur, A.; Landreneau, R. J.; Wu, M.; Swanson, S. J.; Godfrey, T. E.; Litle, V. R. *J. Thorac. Cardiovasc. Surg.* **2008**, *135*(2), 255–260.
b0240 Felli, N.; Fontana, L.; Pelosi, E.; Botta, R.; Bonci, D.; Facchiano, F.; Liuzzi, F.; Lulli, V.; Morsilli, O.; Santoro, S., *et al. Proc. Natl. Acad. Sci. USA* **2005**, *102*(50), 18081–18086.
b0245 Filipowicz, W. *Cell* **2005**, *122*(1), 17–20.
Filipowicz, W.; Pogacic, V. *Curr. Opin. Cell Biol.* **2002**, *14*(3), 319–327. b0250
Flynt, A. S.; Li, N.; Thatcher, E. J.; Solnica-Krezel, L.; Patton, J. G. *Nat. Genet.* **2007**, *39*(2), 259–263. b0255
Forstemann, K.; Horwich, M. D.; Wee, L.; Tomari, Y.; Zamore, P. D. *Cell* **2007**, *130*(2), 287–297. b0260
Forstemann, K.; Tomari, Y.; Du, T.; Vagin, V. V.; Denli, A. M.; Bratu, D. P.; Klattenhoff, C.; Theurkauf, W. E.; Zamore, P. D. *PLoS Biol.* **2005**, *3*(7), e236. b0265
Frankel, L. B.; Christoffersen, N. R.; Jacobsen, A.; Lindow, M.; Krogh, A.; Lund, A. H. *J. Biol. Chem.* **2007**, *283*, 1026–1033. b0270
Fukuda, T.; Yamagata, K.; Fujiyama, S.; Matsumoto, T.; Koshida, I.; Yoshimura, K.; Mihara, M.; Naitou, M.; Endoh, H.; Nakamura, T., *et al. Nat. Cell Biol.* **2007**, *9*(5), 604–611. b0275
Fukushima, T.; Hamada, Y.; Yamada, H.; Horii, I. *J. Toxicol. Sci.* **2007**, *32*(4), 401–409. b0280
Fulci, V.; Chiaretti, S.; Goldoni, M.; Azzalin, G.; Carucci, N.; Tavolaro, S.; Castellano, L.; Magrelli, A.; Citarella, F.; Messina, M., *et al. Blood* **2007**, *109*(11), 4944–4951. b0285
Galardi, S.; Mercatelli, N.; Giorda, E.; Massalini, S.; Frajese, G. V.; Ciafre, S. A.; Farace, M. G. *J. Biol. Chem.* **2007**, *282*(32), 23716–23724. b0290
Gan, J.; Tropea, J. E.; Austin, B. P.; Court, D. L.; Waugh, D. S.; Ji, X. *Cell* **2006**, *124*(2), 355–366. b0295
Garzon, R.; Pichiorri, F.; Palumbo, T.; Iuliano, R.; Cimmino, A.; Aqeilan, R.; Volinia, S.; Bhatt, D.; Alder, H.; Marcucci, G., *et al. Proc. Natl. Acad. Sci. USA* **2006**, *103*(13), 5078–5083. b0300
Georgantas, R. W., III; Hildreth, R.; Morisot, S.; Alder, J.; Liu, C. G.; Heimfeld, S.; Calin, G. A.; Croce, C. M.; Civin, C. I. *Proc. Natl. Acad. Sci. USA* **2007**, *104*(8), 2750–2755. b0305
Gillis, A. J.; Stoop, H. J.; Hersmus, R.; Oosterhuis, J. W.; Sun, Y.; Chen, C.; Guenther, S.; Sherlock, J.; Veltman, I.; Baeten, J., *et al. J. Pathol.* **2007**, *213*(3), 319–328. b0310
Giraldez, A. J.; Cinalli, R. M.; Glasner, M. E.; Enright, A. J.; Thomson, J. M.; Baskerville, S.; Hammond, S. M.; Bartel, D. P.; Schier, A. F. *Science* **2005**, *308*(5723), 833–838. b0315
Giraldez, A. J.; Mishima, Y.; Rihel, J.; Grocock, R. J.; Van Dongen, S.; Inoue, K.; Enright, A. J.; Schier, A. F. *Science* **2006**, *312*(5770), 75–79. b0320
Gironella, M.; Seux, M.; Xie, M. J.; Cano, C.; Tomasini, R.; Gommeaux, J.; Garcia, S.; Nowak, J.; Yeung, M. L.; Jeang, K. T., *et al. Proc. Natl. Acad. Sci. USA* **2007**, *104*(41), 16170–16175. b0325
Gregory, R. I.; Chendrimada, T. P.; Cooch, N.; Shiekhattar, R. *Cell* **2005**, *123*(4), 631–640. b0330
Gregory, R. I.; Yan, K. P.; Amuthan, G.; Chendrimada, T.; Doratotaj, B.; Cooch, N.; Shiekhattar, R. *Nature* **2004**, *432*(7014), 235–240. b0335
Griffiths-Jones, S.; Grocock, R. J.; van Dongen, S.; Bateman, A.; Enright, A. J. *Nucleic Acids Res.* **2006**, *34*(Database issue), D140–D144. b0340
Grimson, A.; Farh, K. K.; Johnston, W. K.; Garrett-Engele, P.; Lim, L. P.; Bartel, D. P. *Mol. Cell* **2007**, *27*(1), 91–105. b0345
Han, J.; Lee, Y.; Yeom, K. H.; Kim, Y. K.; Jin, H.; Kim, V. N. *Genes Dev.* **2004**, *18*(24), 3016–3027. b0350
Harfe, B. D.; McManus, M. T.; Mansfield, J. H.; Hornstein, E.; Tabin, C. J. *Proc. Natl. Acad. Sci. USA* **2005**, *102*(31), 10898–10903. b0355
Hariharan, M.; Scaria, V.; Pillai, B.; Brahmachari, S. K. *Biochem. Biophys. Res. Commun.* **2005**, *337*(4), 1214–1218. b0360
Harris, K. S.; Zhang, Z.; McManus, M. T.; Harfe, B. D.; Sun, X. *Proc. Natl. Acad. Sci. USA* **2006**, *103*(7), 2208–2213. b0365
Hatfield, S. D.; Shcherbata, H. R.; Fischer, K. A.; Nakahara, K.; Carthew, R. W.; Ruohola-Baker, H. *Nature* **2005**, *435*(7044), 974–978. b0370
Hayashita, Y.; Osada, H.; Tatematsu, Y.; Yamada, H.; Yanagisawa, K.; Tomida, S.; Yatabe, Y.; Kawahara, K.; b0375

Sekido, Y.; Takahashi, T. *Cancer Res.* **2005**, *65*(21), 9628–9632.

b0380 He, L.; He, X.; Lim, L. P.; de Stanchina, E.; Xuan, Z.; Liang, Y.; Xue, W.; Zender, L.; Magnus, J.; Ridzon, D., *et al. Nature* **2007a**, *447*(7148), 1130–1134.

b0385 He, L.; He, X.; Lowe, S. W.; Hannon, G. J. *Nat. Rev. Cancer* **2007b**, *7*(11), 819–822.

b0390 He, H.; Jazdzewski, K.; Li, W.; Liyanarachchi, S.; Nagy, R.; Volinia, S.; Calin, G. A.; Liu, C. G.; Franssila, K.; Suster, S., *et al. Proc. Natl. Acad. Sci. USA* **2005a**, *102*(52), 19075–19080.

b0395 He, L.; Thomson, J. M.; Hemann, M. T.; Hernando-Monge, E.; Mu, D.; Goodson, S.; Powers, S.; Cordon-Cardo, C.; Lowe, S. W.; Hannon, G. J., *et al. Nature* **2005b**, *435*(7043), 828–833.

b0400 Hirsch, A. T.; Criqui, M. H.; Treat-Jacobson, D.; Regensteiner, J. G.; Creager, M. A.; Olin, J. W.; Krook, S. H.; Hunninghake, D. B.; Comerota, A. J.; Walsh, M. E., *et al. J. Am. Med. Assoc.* **2001**, *286*(11), 1317–1324.

b0405 Hornstein, E.; Mansfield, J. H.; Yekta, S.; Hu, J. K.; Harfe, B. D.; McManus, M. T.; Baskerville, S.; Bartel, D. P.; Tabin, C. J. *Nature* **2005**, *438*(7068), 671–674.

b0410 Hossain, A.; Kuo, M. T.; Saunders, G. F. *Mol. Cell. Biol.* **2006**, *26*(21), 8191–8201.

b0415 Houbaviy, H. B.; Murray, M. F.; Sharp, P. A. *Dev. Cell* **2003**, *5*(2), 351–358.

b0420 Hsu, P. W.; Lin, L. Z.; Hsu, S. D.; Hsu, J. B.; Huang, H. D. *Nucleic Acids Res.* **2007**, *35*(Database issue), D381–D385.

b0425 Huang, Y. S.; Dai, Y.; Yu, X. F.; Bao, S. Y.; Yin, Y. B.; Tang, M.; Hu, C. X. *J. Gastroenterol. Hepatol.* **2008**, *23*(1), 87–94.

b0430 Hwang, H. W.; Mendell, J. T. *Br. J. Cancer* **2006**, *94*(6), 776–780.

b0435 Hwang, H. W.; Wentzel, E. A.; Mendell, J. T. *Science* **2007**, *315*(5808), 97–100.

b0440 Iorio, M. V.; Ferracin, M.; Liu, C. G.; Veronese, A.; Spizzo, R.; Sabbioni, S.; Magri, E.; Pedriali, M.; Fabbri, M.; Campiglio, M., *et al. Cancer Res.* **2005**, *65*(16), 7065–7070.

b0445 Ji, R.; Cheng, Y.; Yue, J.; Yang, J.; Liu, X.; Chen, H.; Dean, D. B.; Zhang, C. *Circ. Res.* **2007**, *100*(11), 1579–1588.

b0450 Jiang, F.; Ye, X.; Liu, X.; Fincher, L.; McKearin, D.; Liu, Q. *Genes Dev.* **2005**, *19*(14), 1674–1679.

b0455 Jin, Z.; Xie, T. *Curr. Biol.* **2007**, *17*(6), 539–544.

b0460 Joglekar, M. V.; Parekh, V. S.; Hardikar, A. A. *Trends Endocrinol. Metab.* **2007a**, *18*(10), 393–400.

b0465 Joglekar, M. V.; Parekh, V. S.; Mehta, S.; Bhonde, R. R.; Hardikar, A. A. *Dev. Biol.* **2007b**, *311*(2), 603–612.

b0470 Johnson, S. M.; Grosshans, H.; Shingara, J.; Byrom, M.; Jarvis, R.; Cheng, A.; Labourier, E.; Reinert, K. L.; Brown, D.; Slack, F. J. *Cell* **2005**, *120*(5), 635–647.

b0475 Jopling, C. L.; Yi, M.; Lancaster, A. M.; Lemon, S. M.; Sarnow, P. *Science* **2005**, *309*(5740), 1577–1581.

b0480 Kanellopoulou, C.; Muljo, S. A.; Kung, A. L.; Ganesan, S.; Drapkin, R.; Jenuwein, T.; Livingston, D. M.; Rajewsky, K. *Genes Dev.* **2005**, *19*(4), 489–501.

b0485 Ketting, R. F.; Fischer, S. E.; Bernstein, E.; Sijen, T.; Hannon, G. J.; Plasterk, R. H. *Genes Dev.* **2001**, *15*(20), 2654–2659.

b0490 Khvorova, A.; Reynolds, A.; Jayasena, S. D. *Cell* **2003**, *115*(2), 209–216.

b0495 Kim, Y. K.; Kim, V. N. *EMBO J.* **2007**, *26*(3), 775–783.

b0500 Kim, H. K.; Lee, Y. S.; Sivaprasad, U.; Malhotra, A.; Dutta, A. *J. Cell Biol.* **2006**, *174*(5), 677–687.

b0505 Kimura, H.; Kawasaki, H.; Taira, K. *Nucleic Acids Symp. Ser. (Oxf.)* **2004**, *48*, 213–214.

b0510 Kloosterman, W. P.; Lagendijk, A. K.; Ketting, R. F.; Moulton, J. D.; Plasterk, R. H. *PLoS Biol.* **2007**, *5*(8), e203.

b0515 Kluiver, J.; Haralambieva, E.; de Jong, D.; Blokzijl, T.; Jacobs, S.; Kroesen, B. J.; Poppema, S.; van den Berg, A. *Genes Chromosomes Cancer* **2006**, *45*(2), 147–153.

Kok, K. H.; Ng, M. H.; Ching, Y. P.; Jin, D. Y. *J. Biol. Chem.* **2007**, *282*(24), 17649–17657. b0520

Kotaja, N.; Bhattacharyya, S. N.; Jaskiewicz, L.; Kimmins, S.; Parvinen, M.; Filipowicz, W.; Sassone-Corsi, P. *Proc. Natl. Acad. Sci. USA* **2006**, *103*(8), 2647–2652. b0525

Krek, A.; Grun, D.; Poy, M. N.; Wolf, R.; Rosenberg, L.; Epstein, E. J.; MacMenamin, P.; da Piedade, I.; Gunsalus, K. C.; Stoffel, M., *et al. Nat. Genet.* **2005**, *37*(5), 495–500. b0530

Krutzfeldt, J.; Poy, M. N.; Stoffel, M. *Nat. Genet.* **2006**, *38* (Suppl.), S14–S19. b0535

Krutzfeldt, J.; Rajewsky, N.; Braich, R.; Rajeev, K. G.; Tuschl, T.; Manoharan, M.; Stoffel, M. *Nature* **2005**, *438*(7068), 685–689. b0540

Kuehbacher, A.; Urbich, C.; Zeiher, A. M.; Dimmeler, S. *Circ. Res.* **2007**, *101*(1), 59–68. b0545

Kwon, C.; Han, Z.; Olson, E. N.; Srivastava, D. *Proc. Natl. Acad. Sci. USA* **2005**, *102*(52), 18986–18991. b0550

Lagos-Quintana, M.; Rauhut, R.; Lendeckel, W.; Tuschl, T. *Science* **2001**, *294*(5543), 853–858. b0555

Landais, S.; Landry, S.; Legault, P.; Rassart, E. *Cancer Res.* **2007**, *67*(12), 5699–5707. b0560

Landgraf, P.; Rusu, M.; Sheridan, R.; Sewer, A.; Iovino, N.; Aravin, A.; Pfeffer, S.; Rice, A.; Kamphorst, A. O.; Landthaler, M., *et al. Cell* **2007**, *129*(7), 1401–1414. b0565

Landthaler, M.; Yalcin, A.; Tuschl, T. *Curr. Biol.* **2004**, *14*(23), 2162–2167. b0570

Latronico, M. V.; Catalucci, D.; Condorelli, G. *Circ. Res.* **2007**, *101*(12), 1225–1236. b0575

Lau, N. C.; Lim, L. P.; Weinstein, E. G.; Bartel, D. P. *Science* **2001**, *294*(5543), 858–862. b0580

Lecellier, C. H.; Dunoyer, P.; Arar, K.; Lehmann-Che, J.; Eyquem, S.; Himber, C.; Saib, A.; Voinnet, O. *Science* **2005**, *308*(5721), 557–560. b0585

Lee, Y.; Ahn, C.; Han, J.; Choi, H.; Kim, J.; Yim, J.; Lee, J.; Provost, P.; Radmark, O.; Kim, S., *et al. Nature* **2003**, *425*(6956), 415–419. b0590

Lee, R. C.; Ambros, V. *Science* **2001**, *294*(5543), 862–864. b0595

Lee, R. C.; Feinbaum, R. L.; Ambros, V. *Cell* **1993**, *75*(5), 843–854. b0600

Lee, E. J.; Gusev, Y.; Jiang, J.; Nuovo, G. J.; Lerner, M. R.; Frankel, W. L.; Morgan, D. L.; Postier, R. G.; Brackett, D. J.; Schmittgen, T. D. *Int. J. Cancer* **2007**, *120*(5), 1046–1054. b0605

Lee, Y.; Hur, I.; Park, S. Y.; Kim, Y. K.; Suh, M. R.; Kim, V. N. *EMBO J.* **2006**, *25*(3), 522–532. b0610

Lee, Y.; Jeon, K.; Lee, J. T.; Kim, S.; Kim, V. N. *EMBO J.* **2002**, *21*(17), 4663–4670. b0615

Lee, Y.; Kim, M.; Han, J.; Yeom, K. H.; Lee, S.; Baek, S. H.; Kim, V. N. *EMBO J.* **2004a**, *23*(20), 4051–4060. b0620

Lee, Y. S.; Nakahara, K.; Pham, J. W.; Kim, K.; He, Z.; Sontheimer, E. J.; Carthew, R. W. *Cell* **2004b**, *117*(1), 69–81. b0625

Legendre, M.; Ritchie, W.; Lopez, F.; Gautheret, D. *PLoS Comput. Biol.* **2006**, *2*(5), e43. b0630

Leung, A. K.; Calabrese, J. M.; Sharp, P. A. *Proc. Natl. Acad. Sci. USA* **2006**, *103*(48), 18125–18130. b0635

Lewis, B. P.; Burge, C. B.; Bartel, D. P. *Cell* **2005**, *120*(1), 15–20. b0640

Lim, L. P.; Lau, N. C.; Garrett-Engele, P.; Grimson, A.; Schelter, J. M.; Castle, J.; Bartel, D. P.; Linsley, P. S.; Johnson, J. M. *Nature* **2005**, *433*(7027), 769–773. b0645

Lin, S. L.; Chiang, A.; Chang, D.; Ying, S. Y. *RNA* **2008**, *14*, 417–424. b0650

Lindow, M.; Gorodkin, J. *DNA Cell Biol.* **2007**, *26*(5), 339–351. b0655

Lingel, A.; Simon, B.; Izaurralde, E.; Sattler, M. *Nat. Struct. Mol. Biol.* **2004**, *11*(6), 576–577. b0660

Linsley, P. S.; Schelter, J.; Burchard, J.; Kibukawa, M.; Martin, M. M.; Bartz, S. R.; Johnson, J. M.; Cummins, J. M.; Raymond, C. K.; Dai, H., *et al. Mol. Cell. Biol.* **2007**, *27*(6), 2240–2252. b0665

Liu, J.; Carmell, M. A.; Rivas, F. V.; Marsden, C. G.; Thomson, J. M.; Song, J. J.; Hammond, S. M.; b0670

Joshua-Tor, L.; Hannon, G. J. *Science* **2004**, *305*(5689), 1437–1441.
b0675 Liu, Q.; Rand, T. A.; Kalidas, S.; Du, F.; Kim, H. E.; Smith, D. P.; Wang, X. *Science* **2003**, *301*(5641), 1921–1925.
b0680 Liu, J.; Rivas, F. V.; Wohlschlegel, J.; Yates, J. R., III; Parker, R.; Hannon, G. J. *Nat. Cell Biol.* **2005**, *7*(12), 1261–1266.
b0685 Liu, N.; Williams, A. H.; Kim, Y.; McAnally, J.; Bezprozvannaya, S.; Sutherland, L. B.; Richardson, J. A.; Bassel-Duby, R.; Olson, E. N. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 20844–20849.
b0690 Lu, J.; Getz, G.; Miska, E. A.; Alvarez-Saavedra, E.; Lamb, J.; Peck, D.; Sweet-Cordero, A.; Ebert, B. L.; Mak, R. H.; Ferrando, A. A., *et al. Nature* **2005**, *435*(7043), 834–838.
b0695 Lui, W. O.; Pourmand, N.; Patterson, B. K.; Fire, A. *Cancer Res.* **2007**, *67*(13), 6031–6043.
b0700 Lund, E.; Guttinger, S.; Calado, A.; Dahlberg, J. E.; Kutay, U. *Science* **2004**, *303*(5654), 95–98.
b0705 Macrae, I. J.; Zhou, K.; Li, F.; Repic, A.; Brooks, A. N.; Cande, W. Z.; Adams, P. D.; Doudna, J. A. *Science* **2006**, *311*(5758), 195–198.
b0710 Malumbres, M.; Barbacid, M. *Nat. Rev. Cancer* **2003**, *3*(6), 459–465.
b0715 Maniataki, E.; Mourelatos, Z. *Genes Dev.* **2005**, *19*(24), 2979–2990.
b0720 Martello, G.; Zacchigna, L.; Inui, M.; Montagner, M.; Adorno, M.; Mamidi, A.; Morsut, L.; Soligo, S.; Tran, U.; Dupont, S., *et al. Nature* **2007**, *449*(7159), 183–188.
b0725 Martin, M. M.; Lee, E. J.; Buckenberger, J. A.; Schmittgen, T. D.; Elton, T. S. *J. Biol. Chem.* **2006**, *281*(27), 18277–18284.
b0730 Martinez, J.; Patkaniowska, A.; Urlaub, H.; Luhrmann, R.; Tuschl, T. *Cell* **2002**, *110*(5), 563–574.
b0735 Masaki, S.; Ohtsuka, R.; Abe, Y.; Muta, K.; Umemura, T. *Biochem. Biophys. Res. Commun.* **2007**, *364*(3), 509–514.
b0740 McKay, I. A.; Malone, P.; Marshall, C. J.; Hall, A. *Mol. Cell. Biol.* **1986**, *6*(10), 3382–3387.
b0745 Meister, G.; Landthaler, M.; Dorsett, Y.; Tuschl, T. *RNA* **2004**, *10*(3), 544–550.
b0750 Meister, G.; Landthaler, M.; Peters, L.; Chen, P. Y.; Urlaub, H.; Luhrmann, R.; Tuschl, T. *Curr. Biol.* **2005**, *15*(23), 2149–2155.
b0755 Meuwissen, R.; Linn, S. C.; Linnoila, R. I.; Zevenhoven, J.; Mooi, W. J.; Berns, A. *Cancer Cell* **2003**, *4*(3), 181.
b0760 Mi, S.; Lu, J.; Sun, M.; Li, Z.; Zhang, H.; Neilly, M. B.; Wang, Y.; Qian, Z.; Jin, J.; Zhang, Y., *et al. Proc. Natl. Acad. Sci. USA* **2007**, *104*(50), 19971–19976.
b0765 Michael, M. Z.; O'Connor, S. M.; van Holst Pellekaan, N. G.; Young, G. P.; James, R. J. *Mol. Cancer Res.* **2003**, *1*(12), 882–891.
b0770 Miranda, K. C.; Huynh, T.; Tay, Y.; Ang, Y. S.; Tam, W. L.; Thomson, A. M.; Lim, B.; Rigoutsos, I. *Cell* **2006**, *126*(6), 1203–1217.
b0775 Mott, J. L.; Kobayashi, S.; Bronk, S. F.; Gores, G. J. *Oncogene* **2007**, *26*(42), 6133–6140.
b0780 Mourelatos, Z.; Dostie, J.; Paushkin, S.; Sharma, A.; Charroux, B.; Abel, L.; Rappsilber, J.; Mann, M.; Dreyfuss, G. *Genes Dev.* **2002**, *16*(6), 720–728.
b0785 Muljo, S. A.; Ansel, K. M.; Kanellopoulou, C.; Livingston, D. M.; Rao, A.; Rajewsky, K. *J. Exp. Med.* **2005**, *202*(2), 261–269.
b0790 Murchison, E. P.; Stein, P.; Xuan, Z.; Pan, H.; Zhang, M. Q.; Schultz, R. M.; Hannon, G. J. *Genes Dev.* **2007**, *21*(6), 682–693.
b0795 Naguibneva, I.; Ameyar-Zazoua, M.; Polesskaya, A.; Ait-Si-Ali, S.; Groisman, R.; Souidi, M.; Cuvellier, S.; Harel-Bellan, A. *Nat. Cell Biol.* **2006**, *8*(3), 278–284.
b0800 Nakajima, N.; Takahashi, T.; Kitamura, R.; Isodono, K.; Asada, S.; Ueyama, T.; Matsubara, H.; Oh, H. *Biochem. Biophys. Res. Commun.* **2006**, *350*(4), 1006–1012.
b0805 Niu, Z.; Li, A.; Zhang, S. X.; Schwartz, R. J. *Curr. Opin. Cell Biol.* **2007**, *19*(6), 618–627.
b0810 O'Connell, R. M.; Taganov, K. D.; Boldin, M. P.; Cheng, G.; Baltimore, D. *Proc. Natl. Acad. Sci. USA* **2007**, *104*(5), 1604–1609.
b0815 O'Donnell, K. A.; Wentzel, E. A.; Zeller, K. I.; Dang, C. V.; Mendell, J. T. *Nature* **2005**, *435*(7043), 839–843.
b0820 Okamura, K.; Hagen, J. W.; Duan, H.; Tyler, D. M.; Lai, E. C. *Cell* **2007**, *130*(1), 89–100.
b0825 Okamura, K.; Ishizuka, A.; Siomi, H.; Siomi, M. C. *Genes Dev.* **2004**, *18*(14), 1655–1666.
b0830 Oppenheim, A. B.; Kornitzer, D.; Altuvia, S.; Court, D. L. *Prog. Nucleic Acid Res. Mol. Biol.* **1993**, *46*, 37–49.
b0835 Ota, A.; Tagawa, H.; Karnan, S.; Tsuzuki, S.; Karpas, A.; Kira, S.; Yoshida, Y.; Seto, M. *Cancer Res.* **2004**, *64*(9), 3087–3095.
b0840 Ozen, M.; Creighton, C. J.; Ozdemir, M.; Ittmann, M. *Oncogene* **2007**, *27*, 1788–1793.
b0845 Pedersen, I. M.; Cheng, G.; Wieland, S.; Volinia, S.; Croce, C. M.; Chisari, F. V.; David, M. *Nature* **2007**, *449*(7164), 919–922.
b0850 Peters, L.; Meister, G. *Mol. Cell* **2007**, *26*(5), 611–623.
b0855 Plaisance, V.; Abderrahmani, A.; Perret-Menoud, V.; Jacquemin, P.; Lemaigre, F.; Regazzi, R. *J. Biol. Chem.* **2006**, *281*(37), 26932–26942.
b0860 Poliseno, L.; Tuccoli, A.; Mariani, L.; Evangelista, M.; Citti, L.; Woods, K.; Mercatanti, A.; Hammond, S.; Rainaldi, G. *Blood* **2006**, *108*(9), 3068–3071.
b0865 Poy, M. N.; Eliasson, L.; Krutzfeldt, J.; Kuwajima, S.; Ma, X.; Macdonald, P. E.; Pfeffer, S.; Tuschl, T.; Rajewsky, N.; Rorsman, P., *et al. Nature* **2004**, *432*(7014), 226–230.
b0870 Provost, P.; Silverstein, R. A.; Dishart, D.; Walfridsson, J.; Djupedal, I.; Kniola, B.; Wright, A.; Samuelsson, B.; Radmark, O.; Ekwall, K. *Proc. Natl. Acad. Sci. USA* **2002**, *99*(26), 16648–16653.
b0875 Pulciani, S.; Santos, E.; Long, L. K.; Sorrentino, V.; Barbacid, M. *Mol. Cell. Biol.* **1985**, *5*(10), 2836–2841.
b0880 Rajewsky, N. *Nat. Genet.* **2006**, *38* (Suppl.), S8–S13.
b0885 Rao, P. K.; Kumar, R. M.; Farkhondeh, M.; Baskerville, S.; Lodish, H. F. *Proc. Natl. Acad. Sci. USA* **2006**, *103*(23), 8721–8726.
b0890 Raveche, E. S.; Salerno, E.; Scaglione, B. J.; Manohar, V.; Abbasi, F.; Lin, Y. C.; Fredrickson, T.; Landgraf, P.; Ramachandra, S.; Huppi, K., *et al. Blood* **2007**, *109*(12), 5079–5086.
b0895 Rehwinkel, J.; Behm-Ansmant, I.; Gatfield, D.; Izaurralde, E. *RNA* **2005**, *11*(11), 1640–1647.
b0900 Reinhart, B. J.; Slack, F. J.; Basson, M.; Pasquinelli, A. E.; Bettinger, J. C.; Rougvie, A. E.; Horvitz, H. R.; Ruvkun, G. *Nature* **2000**, *403*(6772), 901–906.
b0905 Rinaldi, A.; Poretti, G.; Kwee, I.; Zucca, E.; Catapano, C. V.; Tibiletti, M. G.; Bertoni, F. *Leuk. Lymphoma* **2007**, *48*(2), 410–412.
b0910 Robertson, H. D.; Webster, R. E.; Zinder, N. D. *J. Biol. Chem.* **1968**, *243*(1), 82–91.
b0915 Rodriguez, A.; Griffiths-Jones, S.; Ashurst, J. L.; Bradley, A. *Genome Res.* **2004**, *14*(10A), 1902–1910.
b0920 Rodriguez, A.; Vigorito, E.; Clare, S.; Warren, M. V.; Couttet, P.; Soond, D. R.; van Dongen, S.; Grocock, R. J.; Das, P. P.; Miska, E. A., *et al. Science* **2007**, *316*(5824), 608–611.
b0925 Roldo, C.; Missiaglia, E.; Hagan, J. P.; Falconi, M.; Capelli, P.; Bersani, S.; Calin, G. A.; Volinia, S.; Liu, C. G.; Scarpa, A., *et al. J. Clin. Oncol.* **2006**, *24*(29), 4677–4684.
b0930 Rosenberg, M. I.; Georges, S. A.; Asawachaicharn, A.; Analau, E.; Tapscott, S. J. *J. Cell Biol.* **2006**, *175*(1), 77–85.
b0935 Ruby, J. G.; Jan, C. H.; Bartel, D. P. *Nature* **2007**, *448*(7149), 83–86.
b0940 Saito, K.; Ishizuka, A.; Siomi, H.; Siomi, M. C. *PLoS Biol.* **2005**, *3*(7), e235.
b0945 Saito, Y.; Liang, G.; Egger, G.; Friedman, J. M.; Chuang, J. C.; Coetzee, G. A.; Jones, P. A. *Cancer Cell* **2006**, *9*(6), 435–443.

b0950 Saleh, M. C.; Van Rij, R. P.; Andino, R. *Virus Res.* **2004**, *102*(1), 11–17.

b0955 Schratt, G. M.; Tuebing, F.; Nigh, E. A.; Kane, C. G.; Sabatini, M. E.; Kiebler, M.; Greenberg, M. E. *Nature* **2006**, *439*(7074), 283–289.

b0960 Schwarz, D. S.; Hutvagner, G.; Du, T.; Xu, Z.; Aronin, N.; Zamore, P. D. *Cell* **2003**, *115*(2), 199–208.

b0965 Shan, Y.; Zheng, J.; Lambrecht, R. W.; Bonkovsky, H. L. *Gastroenterology* **2007**, *133*(4), 1166–1174.

b0970 Si, M. L.; Zhu, S.; Wu, H.; Lu, Z.; Wu, F.; Mo, Y. Y. *Oncogene* **2007**, *26*(19), 2799–2803.

b0975 Smirnova, L.; Grafe, A.; Seiler, A.; Schumacher, S.; Nitsch, R.; Wulczyn, F. G. *Eur. J. Neurosci.* **2005**, *21*(6), 1469–1477.

b0980 Sokol, N. S.; Ambros, V. *Genes Dev.* **2005**, *19*(19), 2343–2354.

b0985 Song, J. J.; Liu, J.; Tolia, N. H.; Schneiderman, J.; Smith, S. K.; Martienssen, R. A.; Hannon, G. J.; Joshua-Tor, L. *Nat. Struct. Biol.* **2003**, *10*(12), 1026–1032.

b0990 Sonoki, T.; Iwanaga, E.; Mitsuya, H.; Asou, N. *Leukemia* **2005**, *19*(11), 2009–2010.

b0995 Subramanian, S.; Lui, W. O.; Lee, C. H.; Espinosa, I.; Nielsen, T. O.; Heinrich, M. C.; Corless, C. L.; Fire, A. Z.; van de Rijn, M. *Oncogene* **2007**, *27*, 2015–2026.

b1000 Suh, M. R.; Lee, Y.; Kim, J. Y.; Kim, S. K.; Moon, S. H.; Lee, J. Y.; Cha, K. Y.; Chung, H. M.; Yoon, H. S.; Moon, S. Y., *et al. Dev. Biol.* **2004**, *270*(2), 488–498.

b1005 Sun, Q. A.; Wu, Y.; Zappacosta, F.; Jeang, K. T.; Lee, B. J.; Hatfield, D. L.; Gladyshev, V. N. *J. Biol. Chem.* **1999**, *274*(35), 24522–24530.

b1010 Taganov, K. D.; Boldin, M. P.; Chang, K. J.; Baltimore, D. *Proc. Natl. Acad. Sci. USA* **2006**, *103*(33), 12481–12486.

b1015 Takamizawa, J.; Konishi, H.; Yanagisawa, K.; Tomida, S.; Osada, H.; Endoh, H.; Harano, T.; Yatabe, Y.; Nagino, M.; Nimura, Y., *et al. Cancer Res.* **2004**, *64*(11), 3753–3756.

b1020 Tang, F.; Kaneda, M.; O'Carroll, D.; Hajkova, P.; Barton, S. C.; Sun, Y. A.; Lee, C.; Tarakhovsky, A.; Lao, K.; Surani, M. A. *Genes Dev.* **2007**, *21*(6), 644–648.

b1025 Tang, G. Q.; Maxwell, E. S. *Genome Res.* **2007**, *18*, 104–112.

b1030 Tazawa, H.; Tsuchiya, N.; Izumiya, M.; Nakagama, H. *Proc. Natl. Acad. Sci. USA* **2007**, *104*(39), 15472–15477.

b1035 Teleman, A. A.; Maitra, S.; Cohen, S. M. *Genes Dev.* **2006**, *20*(4), 417–422.

b1040 Thomson, J. M.; Newman, M.; Parker, J. S.; Morin-Kensicki, E. M.; Wright, T.; Hammond, S. M. *Genes Dev.* **2006**, *20*(16), 2202–2207.

b1045 Tomari, Y.; Du, T.; Zamore, P. D. *Cell* **2007**, *130*(2), 299–308.

b1050 Tomari, Y.; Matranga, C.; Haley, B.; Martinez, N.; Zamore, P. D. *Science* **2004**, *306*(5700), 1377–1380.

b1055 Tran, N.; McLean, T.; Zhang, X.; Zhao, C. J.; Thomson, J. M.; O'Brien, C.; Rose, B. *Biochem. Biophys. Res. Commun.* **2007**, *358*(1), 12–17.

b1060 Valencia-Sanchez, M. A.; Liu, J.; Hannon, G. J.; Parker, R. *Genes Dev.* **2006**, *20*(5), 515–524.

b1065 van Rij, R. P.; Andino, R. *Trends Biotechnol.* **2006**, *24*(4), 186–193.

b1070 Varghese, J.; Cohen, S. M. *Genes Dev.* **2007**, *21*(18), 2277–2282.

b1075 Visone, R.; Russo, L.; Pallante, P.; De Martino, I.; Ferraro, A.; Leone, V.; Borbone, E.; Petrocca, F.; Alder, H.; Croce, C. M., *et al. Endocr. Relat. Cancer* **2007**, *14*(3), 791–798.

b1080 Vo, N.; Klein, M. E.; Varlamova, O.; Keller, D. M.; Yamamoto, T.; Goodman, R. H.; Impey, S. *Proc. Natl. Acad. Sci. USA* **2005**, *102*(45), 16426–16431.

b1085 Volinia, S.; Calin, G. A.; Liu, C. G.; Ambs, S.; Cimmino, A.; Petrocca, F.; Visone, R.; Iorio, M.; Roldo, C.; Ferracin, M., *et al. Proc. Natl. Acad. Sci. USA* **2006**, *103*(7), 2257–2261.

b1090 Voorhoeve, P. M.; le Sage, C.; Schrier, M.; Gillis, A. J.; Stoop, H.; Nagel, R.; Liu, Y. P.; van Duijse, J.; Drost, J.; Griekspoor, A., *et al. Adv. Exp. Med. Biol.* **2007**, *604*, 17–46.

b1095 Wang, B.; Love, T. M.; Call, M. E.; Doench, J. G.; Novina, C. D. *Mol. Cell* **2006**, *22*(4), 553–560.

b1100 Wang, Y.; Medvid, R.; Melton, C.; Jaenisch, R.; Blelloch, R. *Nat. Genet.* **2007**, *39*(3), 380–385.

b1105 Weber, M. J. *FEBS J.* **2005**, *272*(1), 59–73.

b1110 Welch, C.; Chen, Y.; Stallings, R. L. *Oncogene* **2007**, *26*(34), 5017–5022.

b1115 Wienholds, E.; Koudijs, M. J.; van Eeden, F. J.; Cuppen, E.; Plasterk, R. H. *Nat. Genet.* **2003**, *35*(3), 217–218.

b1120 Wightman, B.; Ha, I.; Ruvkun, G. *Cell* **1993**, *75*(5), 855.

b1125 Wilfred, B. R.; Wang, W. X.; Nelson, P. T. *Mol. Genet. Metab.* **2007**, *91*(3), 209–217.

b1130 Wu, L.; Fan, J.; Belasco, J. G. *Proc. Natl. Acad. Sci. USA* **2006**, *103*(11), 4034–4039.

b1135 Wu, H.; Xu, H.; Miraglia, L. J.; Crooke, S. T. *J. Biol. Chem.* **2000**, *275*(47), 36957–36965.

b1140 Xiao, C.; Calado, D. P.; Galler, G.; Thai, T. H.; Patterson, H. C.; Wang, J.; Rajewsky, N.; Bender, T. P.; Rajewsky, K. *Cell* **2007**, *131*(1), 146–159.

b1145 Xu, P.; Vernooy, S. Y.; Guo, M.; Hay, B. A. *Curr. Biol.* **2003**, *13*(9), 790–795.

b1150 Yan, K. S.; Yan, S.; Farooq, A.; Han, A.; Zeng, L.; Zhou, M. M. *Nature* **2003**, *426*(6965), 468–474.

b1155 Yang, B.; Lin, H.; Xiao, J.; Lu, Y.; Luo, X.; Li, B.; Zhang, Y.; Xu, C.; Bai, Y.; Wang, H., *et al. Nat. Med.* **2007**, *13*(4), 486–491.

b1160 Yang, W. J.; Yang, D. D.; Na, S.; Sandusky, G. E.; Zhang, Q.; Zhao, G. *J. Biol. Chem.* **2005**, *280*(10), 9330–9335.

b1165 Ye, X.; Paroo, Z.; Liu, Q. *J. Biol. Chem.* **2007**, *282*(39), 28373–28378.

b1170 Yi, R.; O'Carroll, D.; Pasolli, H. A.; Zhang, Z.; Dietrich, F. S.; Tarakhovsky, A.; Fuchs, E. *Nat. Genet.* **2006**, *38*(3), 356–362.

b1175 Yi, R.; Qin, Y.; Macara, I. G.; Cullen, B. R. *Genes Dev.* **2003**, *17*(24), 3011–3016.

b1180 Yu, J.; Wang, F.; Yang, G. H.; Wang, F. L.; Ma, Y. N.; Du, Z. W.; Zhang, J. W. *Biochem. Biophys. Res. Commun.* **2006**, *349*(1), 59–68.

b1185 Zeng, Y.; Cullen, B. R. *Methods Mol. Biol.* **2006**, *342*, 49–56.

b1190 Zeng, Y.; Yi, R.; Cullen, B. R. *Proc. Natl. Acad. Sci. USA* **2003**, *100*(17), 9779–9784.

b1195 Zhang, H.; Kolb, F. A.; Brondani, V.; Billy, E.; Filipowicz, W. *EMBO J.* **2002**, *21*(21), 5875–5885.

b1200 Zhang, H.; Kolb, F. A.; Jaskiewicz, L.; Westhof, E.; Filipowicz, W. *Cell* **2004**, *118*(1), 57–68.

b1205 Zhang, H. H.; Wang, X. J.; Li, G. X.; Yang, E.; Yang, N. M. *World J. Gastroenterol.* **2007**, *13*(20), 2883–2888.

b1210 Zhao, Y.; Ransom, J. F.; Li, A.; Vedantham, V.; von Drehle, M.; Muth, A. N.; Tsuchihashi, T.; McManus, M. T.; Schwartz, R. J.; Srivastava, D. *Cell* **2007**, *129*(2), 303–317.

b1215 Zhao, Y.; Samal, E.; Srivastava, D. *Nature* **2005**, *436*(7048), 214–220.

b1220 Zhao, Y.; Srivastava, D. *Trends Biochem. Sci.* **2007**, *32*(4), 189–197.

b1225 Zhou, B.; Wang, S.; Mayr, C.; Bartel, D. P.; Lodish, H. F. *Proc. Natl. Acad. Sci. USA* **2007**, *104*(17), 7080–7085.

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Keywords: cancer; cardiovascular diseases; dicer; drosha; microRNA; microRNA biogenesis; pathology; physiology; RISC; toxicology

Abstract

MicroRNAs (miRNAs) are 19- to 26-nucleotide RNAs that regulate gene expression. Over 700 human miRNA genes have been identified, with one report predicting close to 1000. Recent computational methods indicate that up to 92% of human genes may be regulated by miRNA. In this chapter, we discuss recent progress in miRNA biology and their roles in human disease pathogenesis, as well as opportunities and challenges of miRNA-based therapies.