American Journal of Biology and Natural Sciences
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

Role of miRNA125 in Gene Regulation and Therapeutic Potential in Diseases

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Received: 2024, 15, Dec
Accepted: 2025, 21, Jan
Published: 2025, 28, Feb

Annotation: The discovery of microRNAs (miRNAs) has revolutionized our understanding of gene regulation, influencing physiological and pathological processes from embryonic development to cancer progression. Despite extensive research, the mechanisms by which miRNAs modulate these complex systems remain inadequately understood, revealing a significant knowledge gap. This study employs a comprehensive literature review and data analysis to explore the roles of miRNAs in development and disease, with a focus on their potential as biomarkers and therapeutic targets. Findings highlight unique miRNA signatures in various diseases, offering insights into their diagnostic and therapeutic implications. These results underscore the necessity for continued research into miRNA functionality and delivery systems for

disease intervention.

Keywords: microRNAs, gene regulation, biomarkers, therapeutic targets, disease progression.

Introduction and a Brief History

MicroRNAs (miRNAs) are a class of small (~19–24 nucleotides in length), endogenous, evolutionarily conserved RNAs that function as posttranscriptional regulators of gene expression. They primarily function by binding to complementary target sequences in messenger RNA (mRNA) and interfering with the translational machinery, thereby preventing or altering the production of the protein product. Follow-up studies also revealed that in addition to repressing translation, miRNA binding to its target mRNA also triggered the recruitment and association of mRNA decay factors, leading to mRNA destabilization, degradation, and resultant decrease in expression levels. miRNAs were discovered in 1993 by Lee and colleagues in the nematode *Caenorhabditis elegans*. In these organisms, the downregulation of LIN-14 protein was found to be essential for the progression from the first larval stage (L1) to L2. Furthermore, the downregulation of LIN-14 was found to be dependent on the transcription of a second gene called lin-4. Interestingly, the transcribed lin-4 was not translated into a biologically active protein. Instead, it gave rise to 2 small RNAs approximately 21 and 61 nucleotides in length. The longer sequence formed a stem-loop structure and served as a precursor for the shorter RNA. Later this group, along with Wightman et al, found that the smaller RNA had antisense complementarity to multiple sites in the 3′ UTR of lin-14 mRNA. The binding between these complementary regions decreased LIN-14 protein expression without causing any significant change in its mRNA levels. These 2 studies together brought forth a model wherein base pairing occurred between multiple lin-4 small RNAs to the complementary sites in the 3′ UTR of lin-14 mRNA, thereby causing translational repression of lin-14 and subsequent progression from L1 to L2 during *C. elegans* development.

This novel mode of regulating gene expression was first thought to be a phenomenon exclusive to *C. elegans*. In 2000, 2 separate groups discovered that a small RNA, let-7, was essential for the development of a later larval stage to adult in *C. elegans*. More importantly, homologues of this gene were subsequently discovered in many other organisms, including humans. The period that followed was marked by a deluge of information wherein multiple laboratories cloned numerous small RNAs from humans, flies, and worms. These RNAs were noncoding, around 19 to 24 nucleotides in length, and derived from a longer precursor with a stem-loop or fold-back structure. Many were found to be evolutionarily conserved across species and exhibited cell-type specificity. The recognition and confirmation of the existence of these small RNAs, now termed microRNAs (mi-RNAs), led to intense research aimed at identifying new members of this family. This resulted in the discovery of multiple miRNAs across different species of plants and animals. An miRNA registry, named miRBase, set up in 2002 serves as the primary online repository for all potential miRNA sequences, annotation, nomenclature, and target prediction information. The current release (miRBase 20) contains 24 521 entries representing hairpin precursor miRNAs that express 30 424 mature miRNA products in 206 species. The biological significance of a vast majority of annotated miRNAs, however, remains unknown and requires functional validation.

Biogenesis of miRNAs

miRNA genes can vary widely in their location in the genome. Earlier studies had revealed 2 distinct classes of miRNAs: those that originated from overlapping introns of protein coding

transcripts and others that are encoded in exons, underscoring the complexities associated with miRNA maturation. Clusters of miRNA genes that coex-press polycistronically with the potential to be transcribed as a single unit were also discovered. It is estimated that approximately 50% of miRNAs are expressed from non– protein coding transcripts. The rest are mostly located in the introns of coding genes and are generally cotranscribed with their host genes and processed separately. Since this is a rapidly evolving field, there is potential for future developments to significantly overhaul the current understanding of miRNA genesis. Based on current knowledge, it can be stated that genomic regions capable of generating mature functional miRNAs can be present on diverse locations within the genome.

A general overview of the steps involved in miRNA biogenesis is illustrated in Fig. 1. miRNA coding transcripts are initially transcribed by RNA polymerase II as long primary miRNAs (several hundred nucleotides long) with a 5′ guano-sine cap and a 3′ polyadenylated tail. These can be either non-coding or coding (present within the intron of a coding gene). The primary miRNA is then processed into ~70- to 120-nucleotide-long precursor RNA (pre-miRNA) by a multiprotein complex called Microprocessor (Fig. 1A). This complex contains a ~160-kDa nuclear RNase III enzyme called Drosha. This enzyme is highly conserved in animals but not in plants. Drosha dimerizes with another double-stranded RNA (dsRNA) binding protein, called DiGeorge syndrome critical region gene 8 (DGCR8) or Pasha, to form the functional Microprocessor complex (Fig. 1A). The newly transcribed pre-miRNA with a typical 5′ phosphate and ~2-nucleotide 3′ overhang is then exported into the cytoplasm by exportin 5 (Exp-5), a Ran-dependent nuclear transport receptor protein (Fig. 1B). In the cytoplasm, the pre-miRNAs are finally processed into mature ~18- to 23-nucleotide-long duplexes by another RNase III enzyme, Dicer-1, with help from dsRNA-binding proteins, protein kinase RNA activator and transactivation response RNA binding protein (Fig. 1C,D). The 2 miRNA strands are then separated, depending on various factors such as thermodynamic asymmetry of the duplex and stability of base pairing at the 5′ end. One strand, termed the *guide strand*, along with the aforementioned and other RNA binding proteins that include trinucleotide repeat-containing gene 6A (TNRC6A), associates with catalytic Argonaute (AGO) proteins, forming a microribonuclear protein complex (miRNP) called RNA-induced silencing complex (RISC) (Fig. 1E). The miRNA strand with the most unstable base pairing at the 5′ end usually acts as the guide strand, while the strand with stable base pairing at the 5′ end (known as the passenger or miR* strand) is usually degraded or, in rare cases, even associates with AGO proteins, enabling both strands to serve as functional miRNAs. The guide strand directs the complex to the target mRNA through sequence complementarity and causes its translational repression (Fig. 1F). Ago2 proteins have been localized to cytoplasmic bodies called GW/P-bodies (processing bodies), where miRNAs bound to their mRNA targets are believed to be stored for degradation or translational repression (Fig. 1G). However, recent evidence also demonstrates thatmiRNA biogenesiscan be Microprocessor independent. Examples include pre–miRNA-like hairpins named “Mirtrons” formed from spliced and disbranched short hairpin introns, some small nucleolar RNAs (snoRNAs), and endogenous short hairpin RNAs (shRNAs).

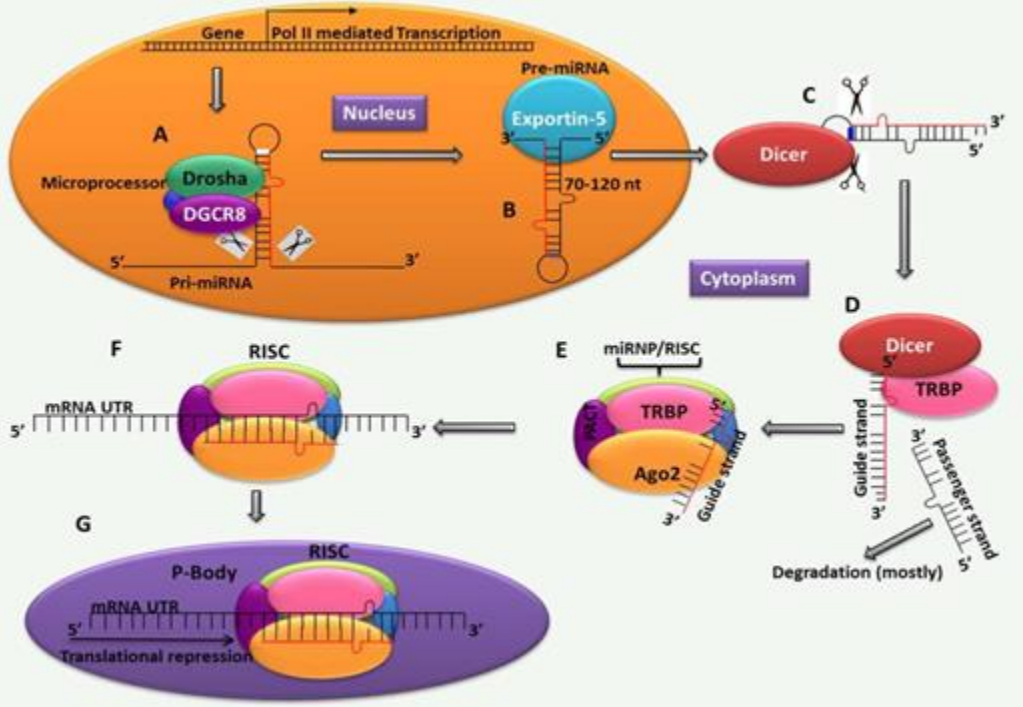

Figure 1

Canonical microRNA (miRNA) biogenesis pathway: miRNAs are initially transcribed as long, variable-length hairpin RNA substrates called primary miRNAs (pri-miRNAs) directly off the DNA template in the nucleus by RNA polymerase II (A). In the nucleus, these...

At least 1 study identified miRNAs to function as activators of target mRNA transcription that play a role in maintaining quiescence during cell cycle, alluding to the possibility that, in rare instances, they can perform dual roles as activators and repressors. Bioinformatic predictions indicate that mammalian miRNAs may regulate up to 30% of all protein coding genes. Emerging evidence also suggests that miRNAs not only play a major role in regulating gene expression but also represent a very critical cellular factor with enormous capability to fine-tune biological processes. In this context, it is remarkable to note that these tiny RNA molecules with such important and ubiquitous roles in regulating cellular processes managed to evade the scientific community's radar for most of the 20th century.

miRNAs in Development and Organogenesis

Animals that do not express miRNAs fail to survive or reproduce normally. The universal impairment of the miRNA pathway by knocking down Dicer in fruit flies and mice caused embryonic lethality with abnormal morphology in almost all organs and resulted in significant lack of stem cells. Embryonic stem cell differentiation, a cardinal event in the development of organs and organ systems, is significantly modulated by miRNAs. Studies show that miRNAs can either promote or inhibit stem cell renewal depending on the cell type or culture environment. In mice, even though Dicer deficiency did not stop the formation of stem cell colonies, it severely impaired the differentiation capability of embryonic stem cells and caused considerable defects in cellular morphology. A subgroup of miRNAs from the miR-290 cluster (cluster implies that these miRNAs are coexpressed from a single transcript) was shown to regulate the embryonic stem cell (ESC) cycle and is now termed the *ESCC family of miRNAs* (ESC cell cycle–promoting miRNAs). ESCC miRNAs directly target the cell cycle inhibitors p21 and LATS2, thus facilitating G1-S phase transition. Moreover, transcription factors such as Oct3/4, Nanog, and Sox2, which are critical for maintaining pluripotency, have been shown to bind to the promoter of the miR-290 cluster and sustain its expression, thereby promoting self-renewal and maintenance of the pluripotent state. ESC miRNA knockout (through deletion of Dicer or Dgcr8) in mouse models resulted in an altered cell cycle profile with an extended G1 phase. As ESCs transition from a self-

renewing to a differentiated state, several ESCC miRNAs show a gradual decrease in expression levels. In contrast, miRNA let-7 acts as a suppressor of pluripotency and is known to antagonize the effects of the miR-290 cluster. Unlike the miR-290 cluster, upregulation of let-7 was detected in the differentiated state, suggesting that its antagonistic effect may help stabilize the differentiated state.

Similarly, miRNAs are also important in regulating the proliferation and differentiation of hematopoietic stem cells. miR-125b performs a specialized role not only in regulating hematopoiesis at the stem cell level but also in modulating inflammation and innate immunity by specifically promoting the differentiation and activation of macrophages. This proinflammatory effect of miR-125b was shown to be mediated predominantly via regulation of the nuclear factor (NF)–κB pathway. Interestingly, the dysregulation of miR-125b has been reported in multiple human cancers, including leukemia, and causes acute myeloid and lymphoid leukemias in mouse models.

miRNAs have been described to play a major role in orchestrating the coordinated development of various organ systems. Although ubiquitously expressed, temporal and spatial expression of distinct sets of tissue-specific miRNAs is important in modeling tissue development and differentiation. miR-273 is required for establishing left-right asymmetry during neuronal development. In mouse heart, the fact that even deletion of 1 of the 2 genes coding for miR-1 (miR-1-2) caused severe and irreparable defects in cardiac morphology suggests critical roles for this miRNA in regulating cardiogenesis. The highly conserved miR-1 is the most abundant miRNA in adult heart, and its known functions include controlling cardiac morphogenesis, electrical conduction, and the cell cycle. miR-1 has been proposed to regulate cardiogenesis by fine-tuning the expression of Hand2, a transcription factor essential for cardiac development. Other validated targets of miR-1 include insulin-like growth factor 1, calmodulin, and myocyte enhancer factor 2A, all of which have been well documented to cumulatively contribute to the development of cardiac hypertrophy. In mice, deletion of miR-208 markedly impaired the ability of the heart to respond to stress stimuli. Double gene knockout of yet another muscle-enriched miRNA, miR-133a, in mice resulted in increased proliferation and apoptosis of myocytes, ventricular septal defects, and embryonic lethality. Those that survived developed severe cardiac dilatation and failure. In skeletal muscle, upregulation of miR-27 and subsequent downregulation of its target protein, Pax3, were found to be important in reducing myocyte proliferation and facilitating myogenic differentiation. Besides cardiac and skeletal muscle, miRNAs also exert specific functions in skin development. miR-203 is induced during differentiation and stratification of mouse skin, which in turn controls the basal to suprabasal transition by repressing p63, a member of the p53 family of transcription factors. On the contrary, p63 represses miR-34a/c expression to maintain cell cycle progression and thus antagonize the effects of miR-203. Along similar lines, expression of miR-124 was found to be essential for proper development of the nervous system.

Systematic expression of miR-127 was found to be essential for proper branching of lung in rat fetal lung cultures. Its untimely overexpression caused defective branching and malformation of lung buds. Transgenic overexpression of the miR-17-92 cluster induced proliferation and inhibited differentiation in lung epithelial progenitor cells. Another important functional role for miRNAs was documented in insulin secretion, where miR-375 expression in pancreatic islets directly altered exocytosis of insulin from pancreatic beta cells. In mice, miR-143 regulates adipocyte differentiation and miR-122 regulates lipid metabolism by altering the expression of multiple target genes. In the proximal convoluted tubules of mouse kidney, miR-192 suppresses Na^+/K^+-ATPase, the major transporter of solutes and fluids in renal epithelial cells, and contributes to renal handling of fluid balance during increased sodium/water intake. There studies, together with the recent developments in high-throughput miRNA profiling that have investigated the spatial and temporal expression patterns in specific organ systems, including whole organisms, have

unequivocally established the role and relevance of miRNAs in animal development and organogenesis.

miRNAs in Diseases

miRNAs in cancer

Apoptosis plays a significant role in both animal development and disease, and the dysregulation of this process has been invariably linked to the progression of various neoplastic processes. miRNAs that regulate apoptosis, termed *apoptomiRs*, can be either pro- or antiapoptotic. The first miRNA described as a regulator of apoptosis was the *Drosophila* gene *bantam*, which directly suppressed the proapoptotic factor *hid*, thus facilitating proliferation. Understandably, many miRNAs that play a role in modulating apoptosis have also been linked to the initiation and progression of various neoplastic processes. Approximately 50% of miRNAs are located at genomic sites that are disrupted or amplified in various cancers. The first evidence of miRNAs playing a role in cancer (termed *oncomiRs*) development came to light in 2002 in a study that attempted to find tumor suppressor genes at chromosome 13q14, which is frequently deleted in chronic lymphocytic leukemia (CLL). CLL is characterized by the presence of substantially increased numbers of predominantly nondividing malignant B cells overexpressing the antiapoptotic B-cell lymphoma 2 (Bcl2) protein. In patients with CLL, the tumor suppressor locus on chromosome 13q14 was found to be frequently altered. However, instead of coding for a tumor suppressor protein, this region contained 2 miRNA genes, miR-15a and miR-16-1, which, when overexpressed, were found to negatively regulate antiapoptotic Bcl2 gene at the posttranscriptional level. Later, many miRNAs that have tumor suppressor roles were identified. The miR-34 family, for example, has been shown to exert significant tumor suppressor capabilities. Upregulation of p53 (a potent tumor suppressor/cell cycle regulator) caused increased miR-34 expression that resulted in G1 arrest in a complementary and parallel fashion to mRNAs that are directly activated by p53. Also, miR-34 was shown to inhibit the silent mating information regulator 1 (SIRT1) gene that resulted in the upregulation of p53, p21, and PUMA (p53-upregulated modulator of apoptosis), thus regulating cell cycle and apoptosis and functioning as a tumor suppressor by modulating the SIRT1-p53 pathway. Furthermore, miR-34 has mediated growth arrest via direct regulation of cell cycle regulatory factors, such as cyclin E2 (CCNE2), cyclin-dependent kinase 4 (CDK4), E2F3, and the hepatocyte growth factor receptor (c-Met), ultimately leading to increased caspase-dependent cell death. In a separate study, miR-34 inhibited the proliferation/growth of human pancreatic tumor–initiating cells, and its overexpression in p53-deficient human pancreatic cancer cells partially restored the tumor-suppressing function of p53. MCL-1, a member of the BCL-2 family, was also demonstrated to be posttranscription-ally regulated by miR-29a, b, and c. Forced expression of miR-29b to induce tumor cell apoptosis by reducing MCL-1 expression may represent a novel intervention for cancer therapy. Along similar lines, let-7a exerts tumor suppressor functions by directly targeting the expression of RAS and HMGA2, 2 widely recognized oncogenes. Other examples of tumor suppressor miRNAs include miR-7, miR-124, miR-137, miR-146b, miR-15b, miR-128, and miR-326. Furthermore, global knockdown of mature miRNAs by selectively targeting Dicer1, RNASEN, and its cofactor DGCR8 increased the oncogenic potential of transformed cell lines, resulting in accelerated tumor formation in mouse models of K-RAS–driven lung cancer and Rb-driven retinoblastoma.

Apart from functioning as tumor suppressors, miRNAs can also promote tumor development (oncogenes) depending on the functions of the target protein(s) they regulate. These oncogenic miRNAs include miR-155 and the miR-17-92 cluster that accelerated tumor development in B-cell lymphomas. Ectopic expression of miR-155 in transgenic mice resulted in pre–B-cell expansion, splenomegaly, and lymphopenia that preceded the development of lymphoblastic leukemia and lymphoma. mir-155 is now known to play a critical role in the development of lymphomas, although the components of its upstream regulatory pathways and downstream targets remain unclear. It is interesting to note that even before the discovery of miRNAs in mammalian cells, Tam et al had reported that the “bic” locus, the common retroviral integration site for the avian

leukosis virus, generated a noncoding RNA. Later, after the discovery of miRNAs, it was found that this transcript harbored the mature miR-155 coding sequence, thus offering a potential explanation for the function of bic. Members of the mir-17-92 cluster are potent activators of cell proliferation and are frequently overexpressed in several neoplasms, including lymphoma, multiple myeloma, medulloblastoma, and cancers of the lung, colon, breast, and prostate. miR-21 is another commonly upregulated miRNA in cancers that include glioblastoma, lymphomas, and cancers of the breast, ovary, colon, rectum, pancreas, lung, liver, gallbladder, prostate, stomach, thyroid, and cervix. Increased expression of miR-21 was found in glioblastoma tumors and cell lines, and its inhibition resulted in increased cell death, suggesting that miR-21 could play the role of an oncogene that inhibited cell death in these tumors. Furthermore, in glioblastoma cells, knockdown of miR-21 induced the activation of caspase-3, transforming growth factor–β, p53, and mitochondrial apoptotic pathways mainly through upregulation of its validated targets, heterogeneous nuclear ribonucleoprotein K, p53-related TAp63, and PDCD4, acting in synergy with the aforementioned proteins.

An individual miRNA can also potentially perform dual functions as an oncogene and tumor suppressor if its targets include antiproliferative genes and growth-promoting genes, respectively. miR-26 functions as an oncogene in glioma and glioblastoma multiforme by regulating PTEN, the molecular antagonist of the Akt pathway, resulting in the inhibition of RB1 and MAP3K2/MEKK2 expression and JNK-dependent apoptosis. miR-26 is also thought to function as a tumor suppressor as its expression was either downregulated or its downregulation resulted in increased anaplasia or metastasis in various neoplasms (eg, hepatocellular carcinoma, breast cancer, squamous cell carcinoma, thyroid cancers, rhabdomyosarcoma, Myc-induced lymphomas) (reviewed in Gao and Liu). More recently, miRNA expression in cancers was shown to be controlled by epigenetic mechanisms such as DNA methylation. In primary lung tumors, methylation of CpG islands in genes for miR-9-1, miR-9-3, miR-34b/c, and miR-193a resulted in their downregulation with a concurrent increase in the expression of their target genes, RAR-β2 and NKIRAS1.[77] Other examples include hypermethylation of miR-91, miR-124a-3, miR-148, miR-152, and miR-663 in human breast cancer and aberrant DNA methylation and downregulation of miR-127 in prostate and bladder cancers. In the latter example, chromatin-modifying drugs successfully restored miR-127 expression and downregulated its predicted target Bcl6, a proto-oncogene, thereby highlighting the therapeutic potential of chromatin-modifying drugs in modulating miRNA expression.

High-throughput techniques such as miRNA microarrays, which provide unique miRNA expression signatures (miRNomes) of different cancers and their subclassifications, are now being used for both diagnostic and classification purposes. miRNomes have proved to be more reliable than mRNA profiles in some tumor subclassifications wherein arrays of ~200 miRNAs provided a better classification of tumors by type and source than a collection of >15 000 mRNAs. Furthermore, miRNAs are more stable than mRNAs in both body fluids and routinely collected formalin-fixed, paraffin-embedded tissues. Recent studies have also detected their presence in plasma and serum of patients (discussed later), thus providing a more convenient and noninvasive approach for miRNA profiling. These developments, coupled with the unique properties of miRNAs, have opened up exciting new avenues for the classification, diagnosis, prognosis, and treatment of various cancers.

miRNAs in infectious diseases

miRNAs can influence the manifestation and pathogenesis of infectious diseases in a multitude of ways. These include modulating the (1) pathogenicity of individual pathogens, (2) the efficiency of host innate and adaptive immune response, and (3) the magnitude and resolution of inflammatory responses. Providing an extensive coverage of the broad range of regulatory roles played by miRNAs in immunity and inflammation is beyond the scope of this review, and the readers are referred to a number of excellent reviews[†] that have dealt with this topic in greater detail. However, it is relevant to note that changes in miRNA expression during

inflammation/immune response are controlled not only by altered transcriptional levels but also by interaction of products of inflammatory/immune responses with the structural and functional components of the miRNA biogenesis pathway.

Studies that have focused on viral pathogenesis pathways revealed that viral infections can alter the levels of host miRNAs that specifically regulate antiviral mechanisms, viral latency, and lytic cycles. These miRNAs either directly target the viral RNA or the host cell factors vital for their replication. Several viruses encode their own miRNAs (v-miRNAs) that in turn regulate their production in host cells. v-miRNAs target and downregulate specific host genes, thereby creating a cellular environment that is permissive for virus replication. Also, from an evolutionary standpoint, it is advantageous and faster to generate a miRNA complementary to a new target gene than produce a regulatory protein that performs the same function. Unlike eukaryotic miRNAs, v-miRNAs are generally not conserved. Pioneering studies done in Epstein-Barr virus (EBV) latently infected B cells have shown that several unique v-miRNAs originated from the Bam H1 fragment H open reading frame 1 (BHRF1) and the Bam H1-A region rightward transcript (BART) present in the viral genome. Some of these v-miRNAs are thought to target lytic genes, suppress viral proliferation, and sustain latency. EBV miRNAs have also been detected in various types of lymphomas that tested positive for EBV. Interestingly, EBV is also known to increase its copy numbers in latently infected cells by hijacking the functions of the host cellular miRNAs, particularly miR-155. Other miRNAs that were reported to be upregulated during latency include miR-21, miR-23a, miR-24, miR-27a, and miR-34a, while miR-96 and miR-128a/b were downregulated in lymph nodes of patients with EBV-positive classic Hodgkin lymphoma.

Viruses such as human immunodeficiency virus 1 (HIV-1) have also been reported to suppress miRNA-mediated silencing during replication in host cells. Components of the host miRNA processing machinery—namely, Dicer and Drosha—were found to be essential in inhibiting virus replication both in HIV-1–infected peripheral blood mononuclear cells and latently infected cells. In this study, when Dicer and Drosha were knocked down using small interfering RNAs (siRNAs), virus replication kinetics was enhanced compared with cells transfected with a nonfunctional siRNA. Furthermore, HIV-1 also actively suppressed the expression of the polycistronic miRNA cluster miR-17/92, which enabled efficient viral replication by upregulating its target histone acetyltransferase Tat cofactor, named PCAF. Along similar lines, the HIV-1 Tat protein, a transcriptional activator with a basic RNA binding domain that can inhibit interferon response, actively suppressed miRNA-siRNA processing by interfering with Dicer activity. In addition, cellular miRNAs such as miR-28, miR-125b, miR-150, miR-223, and miR-382, can inhibit HIV-1 replication by binding to complementary sites located within the viral genome. These anti-HIV miRNAs were found to be enriched in resting $CD4^+$ T cells and were thought to contribute to the development of proviral latency. Interestingly, these miRNAs were also found to be differentially expressed between monocytes and macrophages, providing an explanation as to why macrophages and not monocytes are permissive to HIV infection and replication.

Other examples of cellular miRNAs modulating viral expression include targeting of (1) influenza virus replication by miR-323, miR-491, miR-654, and let-7c; (2) primate foamy virus 1 by miR-32; (3) vesicular stomatitis virus by miR-24 and miR-93; (4) hepatitis B virus by miR-125a-5p, miR-199a-3p, and miR-210; and (5) hepatitis C virus (HCV) by miR-196, miR-296, miR-351, miR-431, and miR-448.‡ A unique and unexpected finding in HCV was that a liver-specific miRNA, miR-122, directly targeted the viral RNA sequence to upregu-late virus replication. This finding is very intriguing as it represents a major deviation from the usual norm that host miRNAs generally repress viral replication.

Given the dependence on miR-122 for HCV replication, blockade of miR-122 function using an experimental DNA-based drug SPC3649 (locked nucleic acid–modified antagomir to miR-122) successfully inhibited HCV replication in chimpanzees. SPC3649 administration at the highest dose produced prolonged suppression of HCV viremia in blood and liver without the emergence of escape mutants. Furthermore, the drug successfully alleviated HCV-induced liver pathology

and produced no side effects in treated animals. This drug is currently being evaluated in phase II clinical trials in humans and, due to the lack of any detectable side effects, has great potential to become the first miRNA-based therapeutic intervention for HCV infection.

Various bacterial diseases also cause marked alterations in the expression of miRNAs, especially in cells involved with the immune response. Recent studies have characterized changes in host miRNA expression following infection with a wide array of bacterial organisms that include both extracellular (eg, *Helicobacter pylori*) and intracellular (eg, *Salmonella enterica*) pathogens. Dysregulated miRNAs have also been demonstrated to affectthe severity of sepsis that follows bacterial infections. Upregulation of miR-155 and downregulation of miR-146a resulted in increased severity of sepsis in murine models of endotoxemia. miR-155 repressed SHIP1 and SOCS1, 2 negative regulators of inflammation, while repression of miR-146a led to upregulation of its targets IRAK1 and TRAF6, 2 proinflammatory signaling proteins associated with the TLR/IL-1R pathways. Upregulation of miR-155 has also been shown to potentiate the immune response against *Salmonella typhimurium*.

In *Mycobacterium tuberculosis* infections, a novel host evasion mechanism mediated by miR-99b has been described wherein miR-99b expression was high in infected dendritic cells and macrophages, and its blockade resulted in significantly reduced bacterial growth. This study also found that knockdown of miR-99b resulted in the upregulation of proinflammatory cytokines such as interleukin (IL)–6, IL-12, and IL-1b and augmented tumor necrosis factor–α (TNF-α) and TNFRSF-4 production. A second study showed that miR-21 can be induced after Bacillus Calmette-Guérin (BCG) vaccination via NF-κB activation. miR-21 suppressed IL-12 production by targeting IL-12p35, which in turn impaired anti-mycobacterial T-cell responses and promoted dendritic cell apoptosis by targeting Bcl-2. An analysis of differentially expressed miRNAs that might play a role in the preferential development of a lepromatous form of leprosy over the tuberculoid form found significant enrichment of miR-21 in *Mycobacterium leprae*–infected monocytes in the lepromatous form. In this study, miR-21 directly downregulated TLR2/1-induced CYP27B1 and IL-1b expression and indirectly upregulated IL-10. The end result of these changes was the inhibition of expression of genes encoding 2 vitamin D–dependent antimicrobial peptides, CAMP and DEFB4A, thus providing an effective mechanism for the bacteria to escape from the vitamin D–dependent antimicrobial pathway. This ability of *M. leprae* to upregulate miR-21 is thought to aid in the progression from the self-limiting tuberculoid form to the progressive lepromatous form of leprosy. The miRNA expression profiles of various infectious diseases, including fungal pathogens, are now available, and the functional implications of these profiles, especially in understanding the pathogenic mechanisms and in developing miRNA-based antimicrobial therapeutics, are aggressively pursued areas of research.

miRNAs in other noninfectious diseases

Dysregulation of miRNA expression has been not only associated with the manifestation of developmental defects in various organisms and organ systems as discussed earlier but also implicated in a wide array of other noninfectious diseases, including autoimmune diseases, metabolic disorders, and genetic diseases. miR-155 and miR-326 are overexpressed in human multiple sclerosis (MS). In vivo silencing of these miRNAs in a mouse model of MS—namely, experimental autoimmune encephalomyelitis—demonstrated that their functional relevance could be attributed to their ability to enhance Th17 response and modulate T-cell developmental pathways by affecting the expression of a complex array of targets. Excess Th17 response is thought to play a key role in the manifestation of various autoimmune diseases. Multiple studies have looked at miRNA expression profiles in various tissues from patients with systemic lupus erythematosus (SLE) or animal models of SLE with considerable variation in the miRNA signatures reported between studies. Most notably, miR-146a was significantly decreased in patients with SLE and was strongly associated with clinical disease activity and activation of the type I interferon (IFN) pathway. Reduced expression of miR-146a resulted in aberrant accumulation of its target proteins (STAT1, IRF5, TRAF6, and IRAK1), leading to a long-lasting

hyperactivation of the IFN pathway and disease manifestation. Patients with SLE are known to display aberrant DNA hypomethylation, and elevated expression of 2 miRNAs, miR-21 and miR-148, has been proposed to contribute to the development of SLE through their effects on inhibiting DNA methylation in T cells.

The role of miRNAs as key regulators of metabolism and the therapeutic implications of these miRNAs in treating metabolic disorders are also being investigated. miRNAs are known to play a major role in controlling glucose homeostasis and insulin signaling. Aberrant miRNA expression in cardiometabolic disorders such as obesity, fatty liver disease, insulin resistance, type 2 diabetes, and coronary artery disease highlights their roles in the manifestation of their respective pathologies. miR-122, expressed primarily in the liver, was the first miRNA to be linked to metabolic control. Early studies showed that miR-122 played a significant role in cholesterol and lipid regulation. Even partial silencing of miR-122 in mice resulted in a ~25% to 30% reduction in plasma cholesterol levels, decreased hepatic cholesterol and fatty acid biosynthesis, and an increase in fatty acid β-oxidation. The silencing of miR-122 also resulted in decreased hepatosteatosis in mice fed a high-fat diet. Similar effects of miR-122 silencing on circulating cholesterol levels were also observed in African green monkeys. However, the exact mechanisms underlying cholesterol lowering in response to miR-122 silencing remains unknown as genes in liver that are involved in cholesterol and lipid metabolism do not seem to be direct targets of miR-122. miR-33a is another miRNA known to control cholesterol homeostasis by cooperating with SREBP2, a cholesterogenic transcription factor, to boost intracellular cholesterol levels (reviewed in Rottiers and Naar). Other miRNAs that play important roles in metabolic disorders include (1) miR-29, which activates insulin secretion, and miR-9 and miR-375, which inhibit insulin secretion via their effect on monocarboxylate transporter 1, one cut homeobox 2, sirtuin 1, phosphoinositide-dependent kinase 1, and myotrophin; (2) modulation of insulin signaling in adipose tissue by miR-103 and miR-107 by targeting caveolin 1 and miR-29; and (3) miR-34a in humans and rodent models of hepatic metabolic diseases, including obesity, type 2 diabetes, nonalcoholic fatty liver disease, and nonalcoholic steatohepatitis, most likely through an intricate regulatory network of miR-34a, SIRT1 (a key sensor and regulator of metabolic states), farnesoid X receptor, and p53 (reviewed in Rottiers and Naar[146]). Furthermore, miR-375 and miR-223 are also present in circulating high-density lipoproteins, indicating that disease-associated miRNAs can be transported in lipoprotein particles, thereby exerting their regulatory effect in a paracrine fashion on distant target tissues.

The importance of miRNAs in genetic diseases is evident from their roles in development and organogenesis described earlier, wherein mutation of relevant miRNAs or target genes is associated with manifestation of a defective phenotype. However, only a few studies have established a clear link between miRNAs and specific genetic disorders. An excellent example of point mutations in the mature miRNA sequence playing an etiopathogenic role in a Mendelian disease includes 2 different nucleotide substitutions in the seed region of miR-96 (13G>A and +14C>A) in 2 Spanish families affected by an autosomal dominant form of deafness, DFNA50. A single base change (A>T) in the seed region of miR-96 in mice also resulted in a progressive hearing loss phenotype, providing convincing evidence that loss of miR-96 target gene regulation arising from point mutations in its seed region results in hearing loss phenotype in both human and mouse. Similarly, mutations and subsequent sequence variations can also occur in the 3′ UTR of mRNAs, thereby altering miRNA recognition/binding sites. Such a mechanism has also been demonstrated in an autosomal dominant form of hereditary spastic paraplegia (SPG31), in which 2 different point mutations in the predicted miR-140 binding site on the 3′ UTR of the *REEP1* gene resulted in failure to regulate its putative target gene expression. At least a couple more studies have looked at human genetic diseases characterized by mutations in genes involved in miRNA processing/biogenesis. Mutations in the *DGCR8* gene, a component of the Drosha complex, is present in DiGeorge syndrome, and loss of function of the *FRM1* gene that codes for an RNA binding protein occurs in fragile X syndrome.

miRNAs in Veterinary Medicine

Most miRNA studies are conducted in laboratory animals and cell lines in which the primary objective is to understand their roles in development and diseases specific to humans. Although studies have documented organ- and breed-specific miRNA signatures in various animal species through computational analysis and polymerase chain reaction/microarray profiling, studies that have directly addressed diseases/disorders specific to food, companion, avian, and exotic animals are extremely limited. The handful of studies in this direction is mostly limited to screening of miRNAs in specific conditions, tissues, or species and have not explored deeper into their mechanism of action and target validation. At least 2 miRNAs, miR-17-5p and miR-181a, have been described to be significantly upregulated in canine B- and T-cell lymphomas. Similarly, in canine osteosarcoma samples, an inverse correlation between decreased expression of miR-134 and miR-544 originating from the 14q32 locus and aggressive tumor growth characteristics was observed. These findings are in agreement with those reported in human osteosarcoma. In canine mammary cancers, especially in tubular papillary carcinomas, miR-29b and miR-21 were found to be significantly upregulated. miRNA expression profiling in canine oral melanoma tissues found that decreased expression of miR-203 was associated with a shorter survival time, thereby highlighting its potential as a new prognostic marker for this disease. The same study also showed marked downregulation of miR-203 and miR-205 in canine and human melanoma cell lines. Over-expression of miR-205 significantly inhibited the growth of canine and human melanoma cells in vitro by targeting erbb3, a member of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases. A recent study compared miRNA expression profiles in the serum of Doberman Pinschers with dilated cardiomyopathy to healthy controls. The study detected the expression of a total of 404 miRNAs in serum, of which 22 showed differential expression between the 2 groups. Although none of the differentially expressed miRNAs attained statistical significance, the study certainly highlights the potential of miRNAs to serve as disease biomarkers in veterinary medicine. Another study along similar lines performed in cats detected a significant increase in miR-122 (>40-fold) and miR-139b (>14-fold) in the serum of newly diagnosed diabetic cats when compared with healthy lean cats and cats in diabetic remission.

An interesting study in Texel sheep, renowned for their exceptional meatiness, identified a new class of regulatory mutations that offer some novel insights into the heritability of complex traits. In this study, the GDF8 allele that encoded for the myostatin gene was found to contain a G to A transition in its 3′ UTR, which in turn resulted in the creation of a target site for miR-1 and miR-206, 2 highly expressed miRNAs in skeletal muscle. This resulted in translational inhibition of the myostatin gene, thereby contributing to the development of muscular hypertrophy in this breed. Similar studies to investigate the role of miRNAs in economically important traits such as mam-mary gland development and skeletal muscle and adipose tissue development, which defines meat quality, are being pursued in multiple species and breeds of production livestock.

miR-181, a well-known positive regulator of immune response, was shown to directly impair or even inhibit porcine reproductive and respiratory syndrome virus (PRRSV) infection. Synthetic miR-181 mimics significantly inhibited PRRSV replication in vitro in a specific and dose-dependent manner by binding to a highly conserved region downstream of open reading frame 4 (ORF4) of the viral genomic RNA. Studies that have profiled miRNAs in lung tissues of pigs infected with *Actinobacillus pleuropneumoniae* and porcine epithelial cells infected with pseudorabies have identified several miRNAs that might play roles in immune and inflammatory responses specific to both pathogens.

miR-146a was elevated in the blood of ferrets, horses, and in a human cervical carcinoma cell line (HeLa) infected with Hendra virus (paramyxovirus, genus *Henipavirus*). Blockade of miR-146a function significantly reduced Hendra virus replication in vitro, suggesting a role for this miRNA in Hendra virus replication. This effect was mostly mediated through its target, ring finger protein, a member of the A20 ubiquitin editing complex that negatively regulates NF-κB activity. Increased NF-κB activity is thought to aid in activating and sustaining Hendra virus replication. A

study that looked at specific miRNA profiles of myopathic horses with polysaccharide storage myopathy or recurrent exertional rhabdomyolysis in 2 different breeds indicated that it might be possible to distinguish one form from the other based on unique miRNA profiles. Interestingly, these miRNA profiles were also found to be breed specific.

In birds, several virus-encoded miRNAs were found to be conserved among different field strains of oncogenic Marek's disease virus (MDV1), and their expression has been detected in both lytic and latent infections, including MDV1-derived tumors. This study found that even though each avian herpes-virus had unique sequences, all originated from similar locations on the viral genome. Hence, these miRNAs tended to be clustered in the rapidly evolving repeat regions of the viral genomes. MDV1 and herpesvirus of turkeys (HVT) encode homologs of the host miRNA, miR-221, which targets a gene important in cell cycle regulation. MDV1 also encodes another miRNA (mdv1-miR-M4), which, together with Kaposi sarcoma–associated herpesvirus-encoded miR-K12-11, was verified as functional orthologs of miR-155, a well-characterized miRNA previously linked to lymphoid malignancies and modulation of immune responses. Novel miRNAs were also found to be encoded by duck enteritis virus, the functions of which remain to be determined. In zebrafish, miR-142-3p was found to be essential for hematopoiesis and affected the cardiac cell fate.