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# The role of microRNAs in animal physiology and pathology

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**Abstract.** MicroRNAs are a class of small, evolutionarily conserved, endogenous RNAs, capable of controlling gene expression. MicroRNAs are transcribed by RNA polymerases II and III, generating precursors that undergo a series of cleavage events to form mature microRNA. They play an important regulatory role in animals at the posttranscriptional levels by targeting mRNAs for direct cleavage of mRNAs or repression of mRNA translation. The main biological function of miRNA is the post-translation regulation of cells, like: proliferation and differentiation, cell death, fat metabolism, neuronal patterning and angiogenesis. These molecules are the main regulators of biological features of economic

interest, including body growth, muscle development, signaling transduction, fat deposition, and immunology. In this review, we summarize the existing knowledge about miRNAs synthesis, mechanisms for regulation of the genome their functions in animals physiology and the implications associated with dysfunction and dysregulation.

**Keywords:** miRNA; biomarkers; small RNAs; miRNA based gene regulations.

## Introduction

MicroRNA belongs to the family of small, single-stranded, endogenous regulatory molecules with the length of 18–25 nucleotides. Evolutionarily conserved molecules occur among various species of plants and animals, including humans [1–3]. Precursors of mature microRNA molecules are short hairpin RNA sequences (shRNA). The main function of miRNA is the post-translational regulation of gene expression as well as the regulation of cell proliferation and differentiation, apoptosis, angiogenesis and oncogenesis [2, 4]. One microRNA molecule can regulate the expression of thousands of genes. It is estimated that these non-coding molecules account for only 1–5% of the human genome and code least 30% of protein coding genes [5]. The recent development of high-throughput sequencing technologies, computational techniques and bioinformatics algorithms has greatly enhanced research on miRNAs, including identification of regulatory targets and prediction of possible functions [6]. The miRBase database from June 2013 contains 24,521 microRNA loci from 206 species [7].

## Biogenesis of microRNA molecules

The miR genes are accumulated on chromosomes in aggregates, which are subject to the translation process as polycistonal transcriptional subunits[8]. Genes encoding microRNAs usually contain several 10s or even 100s of nucleotides and can be located between sequences that code the

protein and function as independent units as well as in coding sequences. MiR genes are also located in introns, exons and non-translated regions [9, 10] (Figure 1). Thanks to this arrangement, transcripts of miRNA as well as mRNA can be created simultaneously [11].

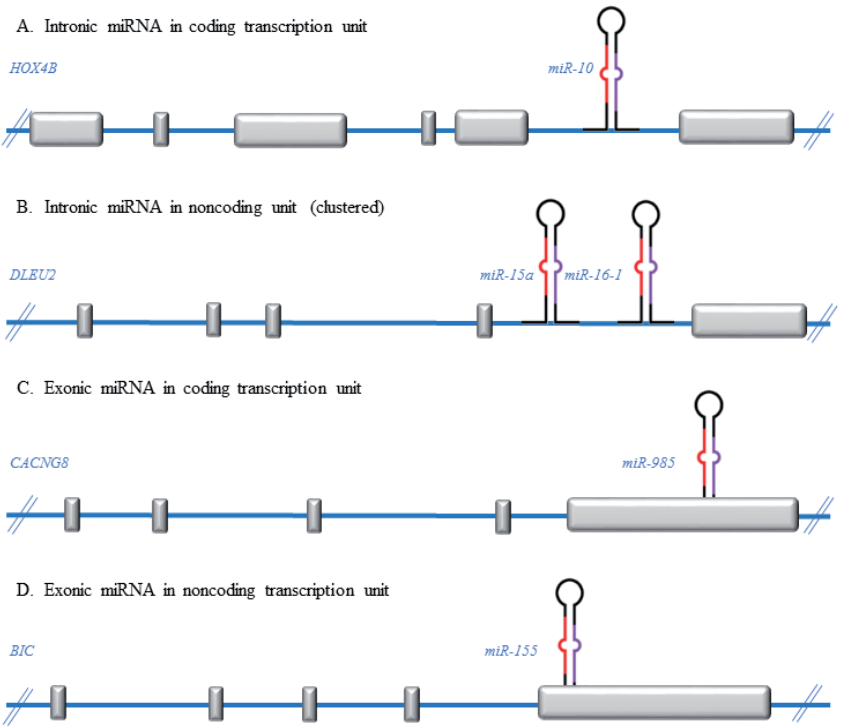


Figure 1. Schematic illustration of the genomic organization and structure of miRNA genes

The conventional biogenesis pathway consists of two cleavage events (one nuclear and one cytoplasmic), and several enzymes play critical roles in the process (Figure 2). The characteristic location of the miRNA genes in the genome enables the action of the RNA polymerase II and III transcribing small RNA [12]. Polymerase II is responsible for the transcription of miRNA

genes in the nucleus and the formation of large, primary miRNA transcripts (pri-miRNA), which, similarly to the mRNA are capped by a 7-methyl guanosine at the 5 ,end and separated by a polyadenyl tail at the 3 ,end [13]. They form specific hairpin-shaped stem-loop secondary structure. Pri-miRNAs reach the length of several base pairs. The double-strand structures of the primary transcript are recognized by the nuclear protein DGCR8/Pasha (a protein contains two double-stranded associated RNA binding domains) with Drosha ribonuclease (RNase III) [14, 15]. The resulting complex participates in the nucleus of the primary miRNA transcript in the nucleus, resulting in a 70 nt pre-miRNA [16].

Pre-miRNA molecules are transported via the exportin 5 (a member of the Ran transport receptor family) interacting with the GTP-dependent Ras protein to the cytoplasm [17, 18]. There, the pre-miRNA processing by the Dicer enzyme, belonging, (like exportin), to RNase III, leads to the formation of about 20–22 nucleotide length double strand miRNA:miRNA duplex with 5' phosphate and a 3' 2 nt overhang from the end of the hairpin structure stem [19, 20]. Duplex is unwound by helicase into two single strands (inactive miRNA is degraded by an unknown enzyme nuclease). Active miRNA mature strand is incorporated into the RISC complex (microRNA induced silencing complex), made up of many proteins, of which Ago proteins play the most important role [21, 22]. The created complex allows for the identification of the passenger thread and the degradation of the second thread. A mature microRNA is created and it contains a conserved sequence of 7 nt at the 5'end significant when binding the target mRNA.

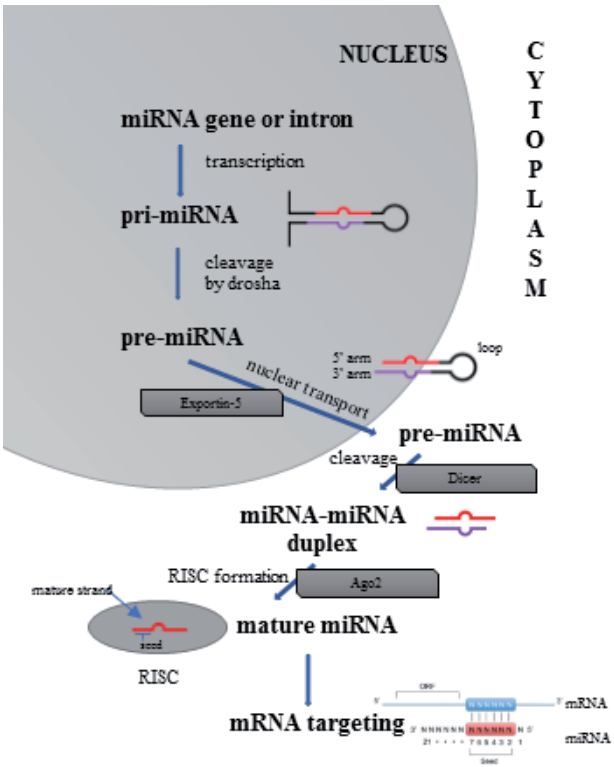


Figure 2. Biogenesis of animal miRNA

## Mechanism of action of microRNA

All miRNAs regulate gene expression at the posttranscriptional level. Three base mechanisms have been described for miRNA-mediated gene regulation: mRNA degradation, translational repression, and miRNA-mediated mRNA decay (Figure 3). One of the ways was defined as the post transcriptional gene silencing (PTGS). It occurs in the case of complete complementarity between miRNA and mRNA, and consists in the hydrolysis of the target mRNA by the enzyme Dicer, and consequently the decrease in the level of a given transcript and the protein encoded by

it [23]. The second method is not dependent on complete complementarity and is manifested by a complete inhibition of the translation process, by linking the miRNA molecule to the mRNA usually located in the 3'UTR region (some miRNAs can also bind to the 5'UTR and/or the ORF) [23–25]. Transcripts are stored or degraded in P bodies found in the cytoplasm, and their level does not change. The mechanism of muting gene expression by inhibiting translation is the mechanism most commonly used in mammalian organisms. A majority of miRNAs downregulate gene expression by translational repression.

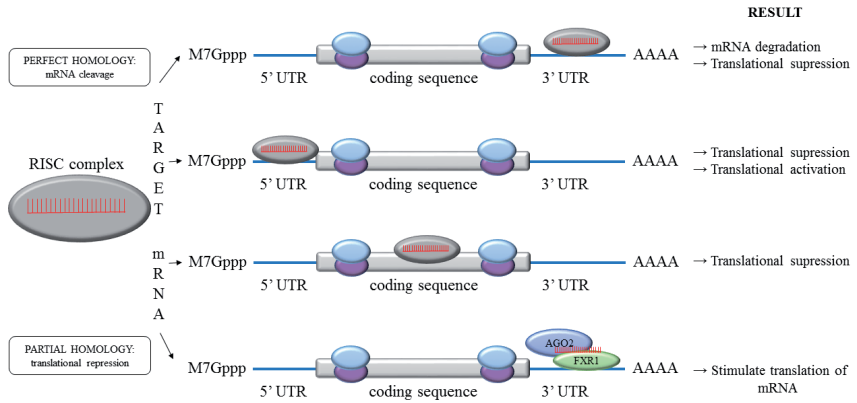


Figure 3. Possible mechanisms for miRNA gene regulation

## MicroRNA as a biomarker

The action of microRNA is based mainly on the regulation of gene expression by complementary linking of base pairs with the target messenger RNA molecule. There is no need for 100% complementarity to the proper functioning of the regulatory mechanism. In the biological processes microRNA plays a huge role in controlling the change of cell proliferation, their differentiation, growth and programmed death – apoptosis. Some microRNA molecules are capable of modifying histones and methylation

of DNA, i.e. changes at the epigenetic level [26–29], which can eventually lead to the formation of pathology process. MicroRNA also affects many physiological and pathological processes in mammalian organisms. Among them, the differentiation of hematopoietic stem cells, skeletal muscle cells, embryogenesis, neurogenesis, angiogenesis or exocytosis is distinguished. They affect the regulation of insulin secretion, pancreatic development, the formation of adipocytes and cells of the immune system. The altered expression of microRNAs is observed in inflammatory conditions, cardiovascular diseases and in viral and bacterial infection. The change in the level of expression of a specific microRNA is associated with the line and the developmental stage of the cell [30–34]. In addition, in pathological conditions, small RNA is released into the blood circulation, becoming a circulating miRNA. Various types of microvesicles, exosomes or apoptotic bodies are found in the circulating microRNA molecules, where they are protected from the degrading effect of RNases [35, 36]. This allows for the use of small, circulating RNAs as stable biomarkers. Circulating microRNAs are also resistant to freezing and thawing processes, thanks to which it is possible to store serum samples at a temperature of –20°C to –80°C without the risk of particle degradation. The fact that microRNAs are good biomarkers is supported by the fact that they are detected in readily available biological fluids, such as saliva, amniotic fluids, urine, and milk [37, 38]. Importantly, they can be identified by specific and sensitive quantitative real time PCR, and most microRNA molecules have been evolutionarily preserved, which facilitates the interpretation of results obtained from animal models in vivo. The expression profiles of circulating microRNAs of healthy individuals are fairly uniform and constant, whereas concentration measurement is possible both in serum and in blood plasma [36, 39].

## Functions of microRNAs in animals

Hundreds of miRNA genes have been found in diverse animals, and many of these are phylogenetically conserved. The biological function of animal

miRNAs has been studied by several approaches [40](Table 1). miRNA molecules are an important regulator of the proper development of many tissues and organs, after each stage of normal growth and development [41], for example: miR-196 in limb [42], miR-133 in heart [43], miR-134 in dendritic spines [44], miR-430 family in brain [45], let -7 in embryo [46, 47] miR-181 in skeletal-muscle differentiation [48] or miR-155 in stem cell mainstance [49].

Table 1. Animal miRNAs and their biological functions (base on [50–52]).

| miRNA | Target(s) | Function(s) | References |
|---|---|---|---|
| Ceanorhabditis elegans | | | |
| lin-4 | lin-14, lin-28 | Physiological condition and developmental timing (larval stage L1 and L2) | [53–55] |
| let-7 | lin-41, HBL-1, DAF-12, PHA-4, RAS | Regulation of late developmental timing (developmental transition from the L2 to the L3 larval stage) | [3, 56–60] |
| lsy-6 | COG-1 | Neuronal cell fate (left/right neuronal asymmetry) | [61] |
| miR-273 | DIE-1 | Neuronal cell fate and developmental timing (left/right neuronal asymmetry) | [62] |
| Drosophila melanogaster | | | |
| bantam | HID | Cell death and proliferation | [63] |
| miR-14 | Drice or caspase | Programmed cell death, proliferation and fat metabolism | [64] |
| miR-7 | Notch targets | Notch signaling | [65, 66] |
| miR-7 | YAN | Photoreceptor differentiation | [67] |
| Danio rerio | | | |
| miR-430 | | Brain morphogenesis | [45] |
| Mus musculus | | | |
| miR-196 | HOXA7, HOXB8, HOXC8, HOXD8 | Developmental patterning | [68] |

Table 1. Animal miRNAs (continued)

| miRNA | Target(s) | Function(s) | References |
|---|---|---|---|
| miR-181 | | Hematopoietic lineage differentiation | [69] |
| miR-1 | HAND2 | Cardiomyocyte differentiation and proliferation | [62] |
| miR-375 | MTPN | Insulin secretion | [70] |
| Rat | | | |
| miR-134 | LIMK1 | Regulation of the size of dendritic spines | [44] |
| Zebrafish | | | |
| miR-430 family | | Brain morphogenesis | [45] |
| let-7 | GFP | Developing embryo | [46, 71, 72] |
| mir-126 | c-MYB | Hemapoetic cell fate | [73] |

First of all miRNAs can regulate developmental timing. Lee et al. [53] demonstrated that lin-4 and let-7, regulate developmental timing in C. elegans. Loss-of-function of this miRNAs result in retarded worm development (lin-4 at the first larval stage, lin-7 at a late stage). miRNA can be involved in regulation of several signaling pathways. Boehm and Slack emphasized a dual function for lin-4, which also play role in regulating life span, possibly through the insulin/insulin-like growth factor-1 pathway [74]. In turn, Li and Carthew demonstrated that the expression of miR-7 is initially triggered by EGF signaling, which results in phosphorylation and inactivation of YAN protein. This changes promote photoreceptor differentiation in D. melanogaster eyes. In literature it is widely described that a miRNA are involved in the regulation of metabolism. Xu et. al suggested that deletion of miR-14 results in animals with increased levels of triacylglycerol and diacylglycerol, whereas increases in miR-14 copy number have the converse effect [75]. In vertebrates, miR-375 is expressed in the pancreatic island and suppresses glucose-induced insulin secretion, what was showed in Poy et al. research [70]. MicroRNA molecules are extremely important regulators of many developmental processes in ani-

mals. Progress in the research methodology allows for the identification of new markers and the attribution of biological role to them.

## MicroRNAs as potential biomarkers for veterinary research

Physiological and pathological roles of miRNA in animal diseases has been the subject of intensive research for only a few years. MicroRNAs are involved in a broad spectrum animal disease, among which the following ones should be mentioned above all: cancer, metabolic and infection diseases and immune defense [76, 77] (Table 2).

Table 2. Selected miRNAs and their function in different domestic animal species.

| miRNA(s) | Significance | References |
|---|---|---|
| Felis domesticus | | |
| miR-122, miR-193b | Diabetes. miRs expression was higher in newly diagnosed diabetic cats compared to healthy lean cats and cats in diabetic remission. | [78] |
| miR-381-3p, miR-486-3p, miR-4751, miR-476c-3p, miR-5700, miR-513a-3p, miR-320e | Hypertrophic cardiomyopathy. Distinct miRNAs and 49 mRNA targets are involved in feline cardiac hypertrophy. | [79] |
| Canis domesticus | | |
| miR-15a, miR-16, miR-29b, miR-21, let-7f, miR-181b | Cancer. miR-15a and miR-16 show a significant downregulation in canine ductal carcinomas while miRsR-181b, -21, -29b, and let-7f show a significant upregulation in canine tubular papillary carcinomas. | [80] |
| mir-29b, miR-101, mir-125a, miR-143, miR-145 | Cancer. Metastatic cells differed in their expression of this set of miRNAs from primary tumors, the comparison of miRNA expression in primary tumors of different malignancy failed to reveal significant differences except for a significant downregulation of mir-125a in metastasizing carcinomas when compared to adenomas. | [81] |

Table 2. Selected miRNAs (continued)

| miRNA(s) | Significance | References |
|---|---|---|
| miR-30b, miR-133b | ACVIM. miR-30b could be a potential biomarker of ACVIM stage B heart failure in Dachshunds with endocardiosis and miR-133b could be a potential biomarker of ACVIM stage C. | [82] |
| Gallus gallus | | |
| miR-122 | Liver metabolism. miR-122 regulates expression of 123 genes in cultured chicken hepatocytes, of which. 21 genes is involved in liver metabolism, so that miR plays role in this process in directly or indirectly. | [83] |
| Set of miRs (esp. miR-2131-5p, miR-221-5p, miR-126-3p, miR-146b-5p, miR-10a-5p, let-7b, miR-125b-5p, and miR-146c-5p, miR-206) | Muscle growth. Most of them are involved in calcium signaling, axonal guidance signaling, and NRF2-mediated oxidative stress response pathways suggesting their involvement in breast muscle growth in chickens. | [84] |
| Sus scrofa domesticus | | |
| Set od miRs | Obesity. miRs were associated mainly with muscle contraction, WNT, mTOR, and MAPK signaling pathways. | [85] |
| miR-203a | FMDV. miR-203a impaired FMDV infection across multiple FMDV serotypes and represent attractive potential of naturally occurring bio-therapeutics against FMDV. | [86] |
| Bos taurus | | |
| miR-125b, miR-141, miRNA-148a, miR-181a, miR-199b, miR-484, miR-500, | Lactogenesis. Systematic predictions differences in types and expression levels of miRNAs providing insight into their possible mechanisms in regulating lactation. | [87–89] |
| Set of miRs | Mastitis. 173 unique miRNAs were identified that had significant differential expression between healthy and mastitis Holstein cattle. Most of them belonged to the chemokine signaling pathway involved in the immune responses. | [88] |
| miR-21, miR-146a, miR-155, miR-222, miR-383 | Mastitis. Inflammation-related miRNA overexpression in the bovine milk was affected by mastitis, and miRNA in milk have potential for use as biomarkers of bovine mastitis. | [90] |

Table 2. Selected miRNAs (continued)

| miRNA(s) | Significance | References |
|---|---|---|
| miR-205, miR-432 | Mycobacterium avium subspecies paratuberculosis. Increased miR-205 and decreased miR-432 expression suggests changes in circulating miRNA profiles due to ageing or development. | [91] |
| Equus caballus | | |
| miR-146a | Hendra virus infection. miR-146a promotes replication of Hendra virus. | [92, 93] |
| miR-140 | Chondrogenesis. miR-140 is an important regulator of cartilage development and homeostasis (through the regulation of CXCL12 and ADAMTS-5) | [94] |

As in humans, the microRNA patterns of different cancers in domestic animals have identified signatures associated with their diagnosis, staging, progression, prognosis, and response to treatment [95]. Boggs et al. [80] determined that miR-29b and miR-21 have a statistically significant up-regulation in canine cancerous samples. In addition, it was observed that miR-15a and miR-16 were down regulated in ductal carcinomas, miR-181b, miR-21, miR-29b, and let-7f were significantly overexpressed in tubular papillary carcinomas diagnosed in dogs. Uhl et al. [96] identified several miRs (including miR19a + b, miR17-5p, miR-203, miR-218 and miR-181a) characteristic for canine lymphoma. Some of them have a predictive, diagnostic, and prognostic potential. In another study, regarding canine osteosarcoma, Leonardo et al. [97] determined a potential role of miR-1 and miR-133b as biomarkers for canine OS treatment. It is worth noting that in many studies in microRNA expression profiling, high molecular homology with human is confirmed.

MicroRNAs play an important role in chondrogenic differentiation. Buechli et al. [94] found that the expression patterns of miR-140 is corelated with cartilage development in horses. In study Peffers et. al [98] increased miR-21 was found in aged horses' articular cartilage. Sumiyoshi et al. [99] shown that overexpression of miR-181a in chicken chondrocytes, directly targets and suppresses the pro-chondrogenic gene (Ccn1)

and aggrecan. In previously study in chicken cells, Kim et al. [100] found, that miR-221 expression increases upon inhibition of chondrocyte differentiation. There are several other miRNAs whose expression is regulated upon chondrocyte differentiation of other species [101–103].

Post-transcriptional gene regulation plays an important role also in infectious diseases [32]. Steward et al. [92] have shown that infection of human cells with HeV changes the expression levels of miR-146a. Up-regulation of this molecule was shown in equine blood experimentally infected with HeV16. They suppose, that miRNA profiling could be used to aid early HeV disease diagnosis, but it's worth remembering that differential expression of miR-146a is induced by several pathogens, including bacteria and other viruses [92, 93, 104–106]. Tian et al. [107] found that miR-15b is a significant marker of Marek's disease virus (MDV) infection in chickens. This miR was reduced in infected susceptible chickens and splenic tumors, controlled by the expression of ATF2. In the another study changes in expression pattern of miR-664-5p, miR-451 and miR-15a were correlated with Actinobacillus pleuropneumoniea [108] and pseudorabies [109] in pigs. That miRs are strongly related to the immune and inflammatory response to both pathogens.

Changes in the microRNA expression level correlate with the manifestation of the functional characteristics of domestic animals. An example of this is the milk yield of cows for which the largest number of tests was carried out. "Milk" miRNAs are transported by exosomes and milk fat globules from mammary gland epithelial cells. The most abundant miRNA found in milk is miRNA-148a. This molecule decreases the expression of gene involved in epigenetic regulation – DNA methyltransferase 1. Another important miRNA of milk is miRNA-125b (targets p53) [89]. Li et. al. [87] in their integrative analysis highlight the complexity of gene expression networks regulated by miRNAs in the bovine mammary gland during lactation. They found a set of genes correlated with lactogenesis, of which miR-125b, miR-141, miR-181a, miR-199b, miR-484 and miR-500 reveal possible biological significance.

## Conclusion

The recent scientific findings in miRNA studies have revealed, that miRNAs are related to cardiac, skeletal muscle and cartilage physiology and pathology. A detailed discussion on all issues exceeds the scope of this study. This review on animal miRNAs shows that these small molecules are great targets for understanding biology, physiology and pathology in veterinary science. In the near future, these molecules may become very attractive features for their immediate implementation as biomarkers for many diseases and may contribute to enhancing global agricultural production as well.

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