Research Progress of microRNA in Ruminants (Postprint)

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Abstract

microRNA (miRNA) is a class of small non-coding RNAs approximately 22 nucleotides in length that regulate mRNA expression by targeting the 3' untranslated region through base pairing, thereby inhibiting translation or promoting degradation. miRNAs are widely present in animals and plants and can participate in the regulation of various developmental and biological processes. Based on a summary of miRNA origin, mechanism of action, prediction methods, and gene expression and function, this article reviews research progress on miRNAs in ruminants.

Full Text

Research Progress of MicroRNA in Ruminants

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Abstract: MicroRNAs (miRNAs) are small non-coding RNA molecules approximately 22 nucleotides in length that regulate mRNA expression through base-pairing with the 3 untranslated region (UTR), thereby inhibiting translation or promoting degradation. Widely present in animals and plants, miRNAs participate in regulating numerous developmental and biological processes. This review summarizes the biogenesis, mechanisms, and prediction methods of miRNAs, along with their gene expression patterns and functions, with a focus on research progress in ruminant animals.

Keywords: microRNA; mRNA; expression; function; ruminant

In recent years, four types of non-coding, single-stranded short RNA molecules have been discovered successively: small interfering RNA (siRNA), Piwi-

interacting RNA (piRNA), microRNA (miRNA), and other small RNAs (eiRNA). All of these regulate cellular functions and biological processes such as tumorigenesis at the post-transcriptional level. With deepening research into gene expression, miRNAs have garnered widespread attention from researchers. As a large family of non-coding RNAs, miRNAs can mediate RNA silencing and regulate organismal life activities through transcriptional and translational modulation. This review focuses on advances in miRNA identification, expression, and functional studies in ruminant animals.

1. Biogenesis and Mechanism of miRNA

miRNAs are small, single-stranded non-coding RNAs of approximately 22 nucleotides that inhibit gene expression through post-transcriptional regulation. miRNAs were initially discovered in *Caenorhabditis elegans*, where lin-4 was found to inhibit protein-coding gene lin-14 expression. Subsequently, hundreds of miRNAs have been identified in various animals and plants, revealing their roles in multiple biological processes including cell proliferation, differentiation, apoptosis, and development.

In eukaryotes, most miRNAs are encoded by their own genes and exhibit high conservation. miRNA maturation involves processing by a series of ribonucleases. The process begins with transcription initiation by RNA polymerase II binding to the miRNA precursor (pre-miRNA) hairpin loop. The primary transcript is then cleaved by the microprocessor complex to form a 70–80 nucleotide precursor containing a stem-loop structure. A rate-limiting step follows, in which pre-miRNA is transported to the cytoplasm by the nuclear transporter Exportin-5. Finally, ribonuclease III (RNase III) removes the stem-loop, retaining a double-stranded miRNA duplex of approximately 20–22 nucleotides. The RNase III-cleavage peptide complex then dissociates the duplex into two single strands. Due to differential stability, the less stable miRNA strand associates with the RNA-induced silencing complex (RISC) to form mature miRNA, while the complementary strand is degraded by nucleases, as illustrated in [Figure 1: see original paper].

2. Prediction and Identification of miRNA Target Genes

miRNA target genes can be predicted computationally. These methods primarily rely on sequence complementarity principles, such as miRanda and TargetScan, while other approaches including PicTar, DIANAmicroT, RNAhybrid, microInspector, RNA22, and SVMicrO employ different computational algorithms [2]. Although the accuracy of these prediction methods is difficult to evaluate, using multiple algorithms to identify common target genes yields substantially higher accuracy than single-software predictions. Predicted miRNA targets require experimental validation, commonly through miRNA overexpression or silencing followed by identification of predicted target genes using quantitative real-time PCR (qRT-PCR) and luciferase reporter assays.

miRNA silencing is typically achieved using antisense oligonucleotides (ASOs), which bind complementarily to miRNAs and prevent base-pairing with target genes. ASOs can be chemically modified through two approaches: 2 -O-methyl antisense oligonucleotides (AMO) and locked nucleic acids (LNA). LNA exhibits higher inhibition efficiency compared to AMO. Another ASO-based approach is miRNA sponging, where RNA sequences transcribed from expression vectors contain multiple tandem binding sites for miRNAs. These sponges competitively sequester miRNAs, thereby preventing their binding to other target genes [3].

Large-scale miRNA target identification can be accomplished through proteomic approaches. For example, selected reaction monitoring (SRM) and isotope-coded affinity tag quantification have identified hundreds of miR-let7 target proteins from wild-type and miR-let7 mutant *C. elegans*, with 161 proteins quantified and 29 showing significant changes between genotypes. SRM results are consistent with downstream analyses including genetic interaction studies, polysome profiling, and luciferase assays, demonstrating that SRM-based miRNA target identification is highly efficient [4].

3. Research Progress of miRNA in Ruminants

miRNA research in cattle has been extensive, with 766 precursor miRNAs processed into 755 mature miRNAs identified through various labeling techniques [5]. Researchers have identified 129 bovine miRNAs from cattle embryos, thymus, small intestine, and lymph nodes, 59 miRNAs from bovine adipose and mammary tissues, and 101 miRNAs from 11 different cattle breeds. Computational predictions from the bovine genome have identified 496 miRNA target genes. More recently, deep sequencing of bovine retinal microvascular endothelial cells identified 255 known bovine miRNAs. Novel miRNA family members such as miR-2284 and miR-2285 have been discovered, which play important roles in angiogenesis [6].

Functional studies of bovine miRNAs have primarily focused on adipose tissue, skeletal muscle, oocytes, and early embryonic development, as summarized in . Jin et al. [14] compared expression of 89 bovine miRNAs in adipose tissues from three hybrid breeds with different backfat thicknesses, identifying 42 differentially expressed miRNAs with miR-378 showing the most significant difference. Since miR-378 plays an important role in rat adipogenesis [15], it may be involved in bovine subcutaneous fat development. miR-143 expression is upregulated during differentiation of bovine intramuscular preadipocytes into mature adipocytes, and transfection of miR-143 complementary sequences inhibits this differentiation [16], indicating its significant role in bovine muscle fat development. Romao et al. [17] identified eight miRNAs expressed in adipose tissue (miR-19a, -92a, -92b, -101, -103, -106, -142-5p, and -296) that correlate with high-fat diets, suggesting their involvement in gene regulatory networks for diet-induced adipogenesis in cattle.

miR-1 and miR-206, two muscle-specific miRNAs, play important roles in mus-

cle cell differentiation [18]. Studies in Piedmontese and Holstein cattle skeletal muscle cells revealed that both miRNAs are expressed and contribute to the double-muscling phenotype caused by a point mutation in the myostatin gene. While miR-1 expression showed no significant difference between breeds or sexes, miR-206 expression was significantly higher in Piedmontese cows than in Holsteins [19], suggesting that upregulated miR-206 may influence muscle hypertrophy in Piedmontese cattle. In Angus cattle subjected to acute stress (rumen fistulation), miR-181b was upregulated in muscle, implying its potential importance in myogenesis [20]. Guo et al. [21] used RNA-Seq to infer that bta-miR-320a may be regulated by transcription factors including SP1 and could affect bovine adipocyte differentiation and fat deposition by targeting genes such as TP53 and mitogen-activated protein kinase 1 (MAPK1). Sun et al. [22] found that miR-10020 can delay early myocyte differentiation or inhibit satellite cell differentiation by suppressing Pax7 gene expression.

Six miRNAs are enriched in oocytes (miR-205, -150, -122, -96, -146a, and -146b-5p), with expression levels decreasing significantly during 0–22 h of oocyte maturation [23], suggesting their roles in bovine oocyte maturation. miR-106a expression is higher in bovine oocytes than in cumulus-oocyte complexes (COCs), while its target gene WEE1A shows lower expression in oocytes than in COCs [24]. These expression differences indicate that miR-106a plays a key role in bovine oocyte development by downregulating WEE1A. Similarly, miR-196a and miR-181a regulate bovine oocyte development by targeting NOBOX and NPM2 genes, which are important for folliculogenesis and nucleolar organization [9-10]. miR-21 and miR-130a expression increases from the 1-cell to 8-cell stage in bovine embryos, suggesting their influence on early embryonic development [25]. Jin [26] found that changes in miRNAs and signaling pathways are associated with abnormal placental development in transgenic cloned cattle.

TABLE:1 Different functions of miRNAs in ruminants

miRNA	Species	Expression location	Target gene	Function	Reference
miR-143	Cattle	Preadipocyte differentiation	-	-	Li et al. [7]
miR-378	Cattle	-	IFNGR1	-	Ma et al. [8]
miR-196a	Cattle	Oocyte	NOBOX	Early embryogenesis	Tripurani et al. [9]
miR-181a	Cattle	Oocyte	-	Early embryogenesis	Lingenfelter et al. [10]
miR-21	Cattle	Retinal microvascular endothelial cells	-	-	Guduric-Fuchs et al. [6]

miRNA	Species	Expression location	Target gene	Function	Reference
miR-22	Sheep	Testicular Sertoli cells	-	Fetal testis development	Torley et al. [11]
miR-1	Sheep	Skeletal muscle cells	-	-	Clop et al. [12]
miR-206	Sheep	Skeletal muscle cells	-	-	Takeda et al. [13]

Several miRNAs (miR-431, -433, -127, -434, -432, and -136) were first identified in double-muscled sheep, revealing their roles in the muscle hypertrophy phenotype. Deep sequencing of skeletal muscle cells from double-muscled sheep identified 747 miRNAs and 472 miRNA precursors [27]. Sheng et al. [28] identified 31 ovine miRNAs using small RNA cloning. A G-to-A mutation in the 3 -UTR of the MSTN gene in Texel sheep creates target sites for miR-1 and miR-206, thereby inhibiting MSTN protein expression and causing muscle hypertrophy. Wang et al. [29] used stem-loop qRT-PCR to detect expression of three miRNAs in sheep longissimus dorsi muscle, finding that oar-miR-299-5p expression was significantly higher in 6-month-old male Hu sheep compared to 6-month-old male Dorper × Hu hybrid sheep, suggesting its involvement in early longissimus dorsi muscle development in Hu sheep. Song et al. [30] detected single nucleotide polymorphisms (SNPs) in the ovine miR-133 precursor sequence, indicating their potential importance for miR-133 precursor processing and their influence on sheep meat production traits.

miRNA microarray analysis detected expression of 159 miRNAs in sheep flank and ear skin, with 19 miRNAs specifically expressed or significantly enriched in flank skin, confirming their roles in wool growth. Sun et al. [31] identified miR-381, -543, -129, and -544 as potentially involved in melanogenesis in goat fetal skin. miR-let7a is expressed in both the growth and regression phases of cashmere goat hair follicle development and represents an important regulatory factor, functioning through target genes Cmyc and FGF5 [32]. Yuan [33] found that miR-125b regulates hair follicle cycling-related genes by inhibiting FGF5 expression, with overexpression affecting mRNA levels of these genes. Yang et al. [34] discovered that lpa-miR-nov-66 can regulate melanin synthesis in melanocytes by binding to the sGC target gene and participating in the cyclic adenosine monophosphate (cAMP) pathway, a conclusion further supported by Ji et al. [35].

Recently, McBride et al. [36] identified 212 ovine miRNAs in ovarian follicles and corpora lutea at different reproductive stages using small RNA library sequencing. Torley et al. [11] compared expression of 128 miRNAs in sheep ovaries and testes at gestational days 42 and 75 using qRT-PCR, finding 24 differentially

expressed miRNAs at day 42 and 43 at day 75. In situ hybridization localized miR-22 to testicular Sertoli cells, suggesting its role in fetal testis development through inhibition of estrogen signaling pathways. Fetal testosterone treatment induces ovarian pathological changes, with miR-497 and miR-15b upregulated in fetal ovaries of testosterone-treated pregnant ewes, indicating their potential involvement in testosterone-induced ovarian pathology [37].

miRNAs play important regulatory roles in biological processes. Hundreds of miRNAs have been identified in various animals, all exhibiting spatiotemporal-specific expression patterns, indicating their crucial functions in specific tissue types or developmental stages. Although functional studies of ruminant miRNAs face many limitations, identification of miRNA target sites remains a breakthrough point, and mRNA-miRNA interactions are essential for studying physiological processes. Recent genome-wide association studies indicate that complex traits are often associated with non-coding SNPs. The role of miRNAs in gene expression may result from associations with these non-coding SNPs, as relevant SNPs may represent partial miRNA binding sites or miRNA sequences. Therefore, further miRNA research should focus on identifying relationships between SNP overlaps with miRNA binding sites or miRNA sequences and growth or reproductive traits in ruminants. Such studies will provide genetic foundations for selecting superior reproductive performance or growth traits in ruminants.

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