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Popular Article

miRNAs in Bovine Genome: An Introduction

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Abstract:

MicroRNAs (miRNAs) are small, endogenous, and evolutionarily conserved RNA molecules that regulate gene expression at the post-transcriptional level by binding to complementary sequences in messenger RNA (mRNA), resulting in translational repression and mRNA degradation. First identified in Caenorhabditis elegans in 1993, miRNAs have since been found to play pivotal roles in diverse biological processes across species, including cattle (Bos taurus). The bovine genome contains over 790 mature miRNAs, many of which are specific to cattle and are involved in the regulation of immune responses, inflammation, and host-pathogen interactions. Despite significant advances in profiling miRNA expression in bovine tissues, particularly in immune-related cells, the functional roles of miRNAs in regulating bovine immunity, particularly during viral infections, remain underexplored. Research to date has largely focused on bacterial infections, such as mastitis, where miRNA expression patterns have been shown to change in response to pathogens like Streptococcus uberis and Staphylococcus aureus. These findings suggest that miRNAs have potential as non-invasive biomarkers for disease detection and monitoring in cattle. Furthermore, miRNAs may serve as promising therapeutic targets for enhancing immune responses or combating infections in livestock. This review highlights the current state of bovine miRNA research, identifies key knowledge gaps, and proposes future directions for investigating miRNA-mediated regulation of immune function, their potential as diagnostic biomarkers, and their therapeutic applications in veterinary medicine.

Introduction:

MicroRNAs (miRNAs) are a type of small (approximately 19–24 nucleotides long), endogenous, and evolutionarily conserved RNA that serve as regulators of gene expression at the post-transcriptional level (Kumar et al., 2013; Srinivasan et al., 2013). Their main role involves binding to complementary sequences

in messenger RNA (mRNA) and disrupting the translational machinery, which inhibits or modifies the synthesis of the protein product (Lawless et al., 2014). Subsequent research has shown that, in addition to inhibiting translation, the interaction between miRNAs and their target mRNA also leads to the recruitment and association of factors that promote mRNA decay, resulting in mRNA destabilization, degradation, and a consequent reduction in expression levels.

MicroRNAs (miRNAs) were first identified in 1993 by Lee and his team in the nematode Caenorhabditis elegans. In these organisms, the inhibition of LIN-14 protein was demonstrated to be crucial for the transition from the initial larval stage (L1) to L2 (Lee *et al*., 1993).

Nomenclature of miRNAs:

A standardized system for naming and labeling has been established to ensure consistency and ease in cataloging miRNAs. miRNAs are assigned numbers sequentially based on the order in which they are identified (Griffiths-Jones *et al*., 2008). Those that have been validated through experimentation receive a number prefixed with "miR," followed by a dash (for example, miR-21). In the identifier hsa-miR-21, the first three letters represent the organism (for example, hsa stands for Homo sapiens). The mature miRNA is referred to as miR-21 (with an uppercase R), while the lowercase mir-21 encompasses both the miRNA gene and the predicted stem-loop structure of the primary transcript, known as the precursor miRNA. Identical mature miRNA sequences that come from different precursor sequences and genomic locations are assigned identifiers with a numeric suffix, such as hsa-miR-219-1 and hsa-miR-219-2. Conversely, closely related mature sequences that vary by 1 or 2 nucleotides are assigned a lettered suffix. This indicates that hsa-miR-130a and hsa-miR-130b are produced from precursors hsa-mir-130a and hsa-mir-130b, respectively.

Recent deep sequencing analyses have shown that single pre-miRNAs can produce multiple mature miRNA species or sequences, which may differ in length or sequence due to various modifications such as trimming at the 5′/3′ ends, substitutions, insertions, deletions, or additions at the 3′ end. These variations, referred to as isomirs, are currently being intensely studied for their biological importance. Additionally, efforts are underway to update the nomenclature system to include these variants (Morin *et al*., 2008). Certain miRNA precursors can generate two mature miRNAs that are approximately 21 to 23 nucleotides long. When the primary miRNA species can be clearly identified, it is referred to by the established name (for example, miR-136), while the one derived from the opposing strand of the precursor is given the same name with an asterisk added (miR-136). However, if it's not possible to determine which species is predominantly expressed, identifiers like miR-502-5p (from the 5′ strand) and miR-502-3p (from the 3′ strand) are used. miRNAs can also exist in clusters and are often located near one another within the genome. For instance, the miR-17 cluster includes six precursor miRNAs that can develop into seven mature miRNAs, including miR-17, miR-91, miR-18, miR-19, miR-19b, miR-20, and miR-92, and are

situated within a 1-kilobase region of chromosome 13 (Lee et al., 2002).These groups have been identified in the literature as either a miR-17-92 cluster, which includes both the lowest and highest numbered miRNA, or a miR-17 cluster, which only includes the smallest numbered miRNA.

Bovine Genome MicroRNAs:

The first studies that demonstrated the presence of miRNA in bovine tissues were carried out in 2007 (Coutinho *et al*., 2007; Gu *et al*., 2007). Since then, 793 mature miRNAs that are encoded in all 30 chromosomes of the Bos taurus genome have been found. Ensembl (v75) estimates that there are 3825 non-coding RNAs in the genome, of which these miRNAs make up about 25% (Flicek *et al*., 2014). According to the common sequence similarity of their seed region, the mature sequence, or precursor miRNA sequence, miRNAs are usually categorized into families (Kozomara et al., 2014). In some genomic areas, clusters of miRNA families are frequently found next to target genes (Wang *et al*., 2011).

Several miRNA families found in the bovine genome appear to be species-specific, particularly when compared to other genomes that are currently accessible. For example, the bta-miR-2284 and bta-miR-2285 families, produce more than 100 mature miRNAs in cattle but do not appear to have human or mouse counterparts. The genes that these miRNAs target are yet unknown, despite studies showing that these miRNA families are expressed in a variety of immune-related bovine tissues, including alveolar macrophages, CD14+ monocytes, and mammary epithelial cells (Lawless *et al*., 2013; Vegh *et al*., 2013).

Role of the miRNAs in the Bovine Immune System:

The roles that miRNAs play in regulating immune activation and resolution in response to infection is less well understood in cattle, compared to human and mouse. Investigations in cattle have primarily focused on characterizing miRNA expression in immune-related and investigating whether miRNA expression is perturbed during bacterial/viral infections – but detailed mechanistic studies are, to date, largely lacking. One of the first studies to profile immune-relevant miRNA expression in cattle, characterized the expression of more than 100 bovine orthologs of known human miRNAs, as well as novel bovine miRNAs, in several immune-relevant tissues and provided an early bovine miRNA expression atlas for many later studies (Coutinho *et al*., 2007). More recently, Vegh et al. utilized a next generation sequencing (NGS) strategy to profile miRNA expression on a genome-wide scale in bovine alveolar macrophages, the primary host cell for M. bovis, an economically important pathogen (Veg *et al*., 2013). NGS has several advantages over classical sequencing technologies, which include the ability to accurately measure expression of all miRNAs simultaneously, with high reliability, single-nucleotide resolution and across the broad dynamic range of expression (Wang *et al*., 2009). miRNA expression has now been demonstrated in 10 immune-related bovine tissues (bovine embryo, thymus, small intestine, mesenteric lymph node, abomasum lymph node, CD14+ blood isolated monocytes, CD14+ milk-isolated monocytes (MIMs), mammary epithelial cells, alveolar macrophages, mammary tissue, and in the MDBK cell line) and

tissue-specific expression of miRNAs in these tissues is readily apparent (Naeem *et al*., 2012; Jin *et al*., 2014).

Several studies have also examined whether miRNA expression is altered in response to Gram-positive or Gram-negative infections associated with bovine mastitis, a disease of the bovine mammary gland with significant economic consequences to the dairy industry. Four of the fourteen miRNAs were found to undergo differential expression. Another study compared transcriptional changes of five inflammation-associated miRNAs, including ones with extensively studied human orthologs: bta-miR-155, bta-miR-146a, and bta-miR-223, in bovine CD14+ monocytes stimulated with either LPS or Staphylococcus aureus enterotoxin B (SEB) (Dilda *et al*., 2012). Four miRNAs were differentially expressed, and LPS was found to have a greater effect than SEB at inducing miRNA differential expression.

Future Research on Bovine miRNAs and Their Impact on Immune Function, Inflammation and Infection:

The findings and recent advancements in miRNA biology have essentially transformed the biological landscape and sparked a renewed interest among scientists in reassessing and significantly changing the accepted ideas regarding gene expression, gene regulation, and RNA function (Rossbach, 2010). The "central dogma of molecular biology," according to which RNA serves just as a channel for information between the genetic material (DNA) and the final product (protein), is effectively challenged by the discovery of miRNA (Robinson, 2009).

It is evident that miRNAs are essential for controlling immunological responses in both humans and mice. Up until now, research on cattle has mostly focused on showing that immune-relevant tissues or cells challenged in vitro with certain pathogens exhibit distinct expression of miRNAs. A bovine miRNA expression atlas across bovine tissues and cells is necessary to close the significant gap in the number of annotated miRNAs in the bovine genome compared to that in the genomes of mice or humans. Improved non-coding RNA annotation has a variety of applications, one of which is to help interpret data from bovine genome-wide association studies (GWAS). For instance, it was recently discovered that the sole gene in a novel genome-wide significant QTL for somatic cell score, a mastitis indicator trait, is a small non-coding RNA that had not been previously annotated (Meredith *et al.,* 2013).

In addition to comprehending the essential biology of how miRNAs control bovine gene expression, miRNAs could also serve as valuable biomarkers for specific diseases in cattle. Certainly, miRNAs have multiple characteristics that have sparked considerable interest in their use as non-invasive biomarkers in human clinical research. miRNAs are highly expressed in various extracellular fluids, remain stable, are simple to detect, and often display specific temporal and spatial characteristics (Chen *et al*., 2010). Small amounts of miRNAs can also have significant information content, making them precise biomarkers. Numerous studies have looked into miRNA expression patterns linked to various mastitis-causing

pathogens, but most were done in lab settings. The use of miRNAs as bovine disease biomarkers is hindered by the lack of in vivo comparisons of miRNA profiles across different pathogens in the same model. In order to pinpoint sensitive and specific biomarkers for specific infections, in vivo studies are essential (Kusuda *et al*., 2011). This has the potential to identify infections, like tuberculosis, through a basic miRNA biomarker, or to differentiate between various infections, such as E. coli and S. uberis-induced mastitis, guiding veterinarians in choosing more targeted treatments.

Another constraint arises from the fact that current research on the impact of miRNAs on bovine immunity has mainly concentrated on bacterial infections, leaving a significant knowledge gap regarding the involvement of miRNAs in bovine viral infections. Several economically significant and prominent bovine infectious diseases stem from viruses, such as Foot and Mouth Disease Virus (FMDV) (Jamal *et al.* , 2013), Bovine Viral Diarrheal Virus (BVDV) (Brodersen, 2014), and the newly identified Schmallenberg virus (Koenraadt *et al.* , 2014). It is evident in various species that host miRNAs play a key role in adjusting the host's immune response to viral infections (Sung and Rice, 2009). Moreover, certain viruses have their own set of miRNAs to evade the immune response of the host. Approximately 200 viral miRNAs have been documented in a study by Skalsky and Cullen in 2010.In addition to their usefulness as biomarkers, miRNAs also show promising prospects as therapeutic targets or agents. MicroRNA function can be enhanced through techniques such as over-expression, involving miRNA mimics or vector-based approaches, or through inhibition, using miRNA sponges or anti-miR oligonucleotides (Van Rooij and Kauppinen, 2014). Numerous miRNAs in humans are currently being tested in preclinical and clinical trials as innovative therapeutics for cancer, viral infections, and cardiovascular disease. An example is the investigation of human miR-122 for its potential therapeutic impact on regulating cholesterol metabolism (Rottiers and Naar, 2012).

Concluding Remarks:

The miRNAs with therapeutic potential mentioned above have orthologs in cattle, indicating the possibility of applying miRNA-based therapeutic strategies to address diseases and regulate metabolism in cattle, potentially influencing key economic traits such as growth, feed efficiency, and milk production (Van Rooij and Kauppinen, 2014). However, a significant challenge in translating miRNA biology to cattle is the lack of validated miRNA targets, as only a few studies have experimentally confirmed the predicted targets (Lawless et al., 2013). Although computational methods can predict hundreds or even thousands of potential miRNA targets, experimental validation is costly and time-consuming. Approaches that integrate miRNA expression data with mRNA expression from the same sample can help refine these predictions and improve the validation success rate. Additionally, recent advancements in technologies like crosslinking immunoprecipitation sequencing (CLIP-Seq), which allows for genome-wide identification of miRNA targets, have yet to be applied to cattle research (Darnell, 2010).

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