Chapter 5

Non-Coding RNA Roles in Ruminant Mammary Gland Development and Lactation

Duy N. Do and Eveline M. Ibeagha-Awemu

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67194

Abstract

The ruminant mammary gland (MG) is an important organ charged with the production of milk for young and human nourishment. Many factors influence MG productivity, including nutrition, genetics, breed, epigenetics (including non-coding RNA [ncRNA]), disease pathogens and other environmental factors. In recent years, increasing research is beginning to determine the role of non-coding RNA in MG functions. Non-coding RNAs (small interfering RNA [siRNA], microRNA [miRNA], PIWI-interacting RNA [piRNA], small nucleolar RNA [snoRNA] and long non-coding RNA [lncRNA]) are a class of untranslated RNA molecules that function to regulate gene expression, associated biochemical pathways and cellular functions and are involved in many biological processes. This chapter presents a review of the current state of knowledge on the role of ncRNAs (particularly miRNAs and lncRNAs) in the MG and lactation processes, lactation signalling pathways, lipid metabolism, MG health of ruminants as well as miRNA roles in milk recipients. Finally, the potential application of new genome editing technology for ncRNA studies in MG development, the lactation process and milk components is presented.

Keywords: non-coding RNA, microRNA, long non-coding RNA, mammary gland, lactation, genome editing, signalling pathways

1. Introduction

As one of the remarkable products of evolution, lactation is a very dynamic and complex process. The process of lactation involves the development of the mammary gland (MG) and the synthesis and secretion of milk. The lactation process is affected by many factors, including genetics, epigenetics, non-genetics and environmental factors. The knowledge of lactation regulation is not only important for improvement of milk production and quality

but also provides a model for basic cellular processes (proliferation, differentiation, survival and death) [1], which may have important implications for productivity (milk yield) and disease status (e.g. breast cancer, mastitis, etc.). The endocrine regulation and physiological processes as well as the signalling pathways involved in these processes are fairly understood [1, 2]. Facilitated by the release of the whole genome sequences of cattle, sheep and goat [3–6] as well as availability of single nucleotide polymorphism (SNP) genotyping chips [7–11], the genetic mechanisms of ruminant lactation have been extensively explored (**Figure 1**). As a consequence, many quantitative trait loci (QTL) and genetic markers for lactation-related traits (for instance, milk yield, milk components, lactation persistency, etc.) have been detected and catalogued in the animal QTL database (http://www.animalgenome.org/cgi-bin/QTLdb/index).

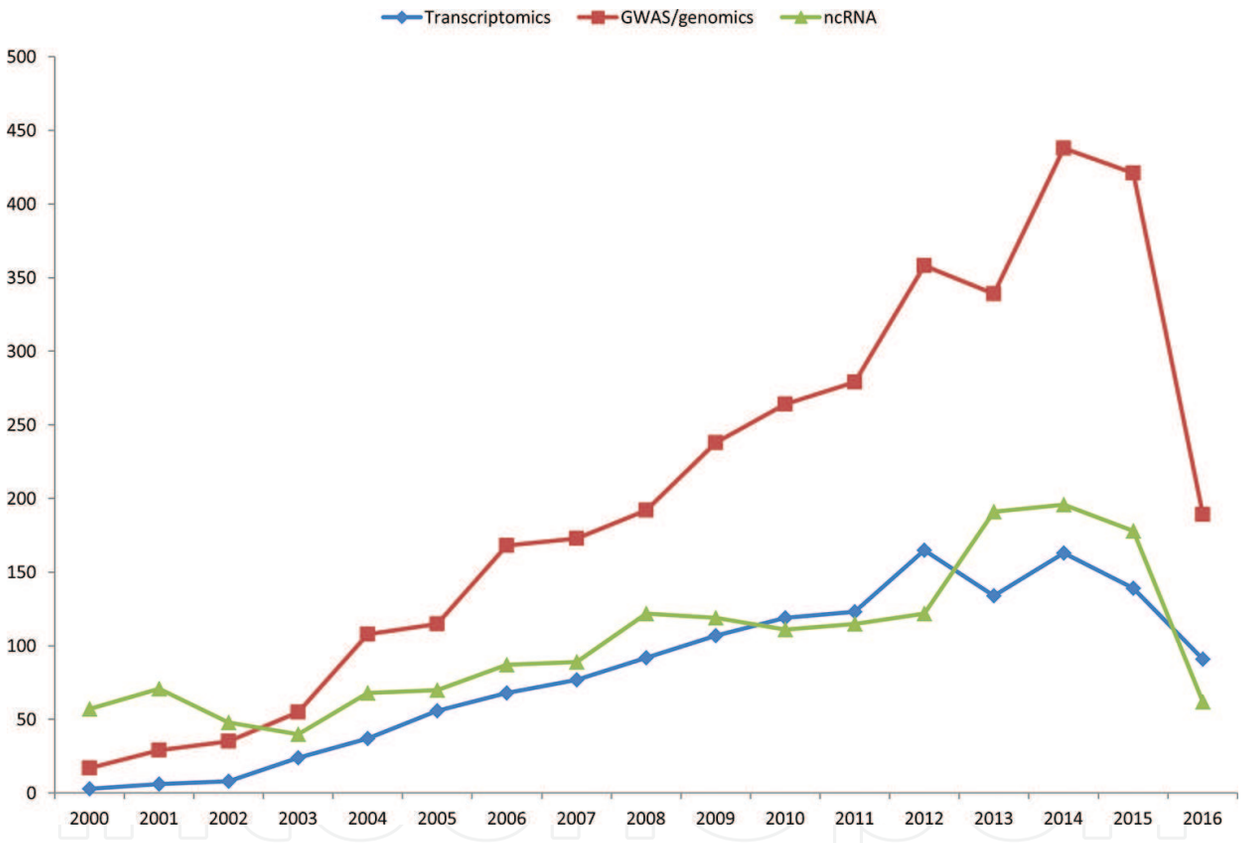

Figure 1. Growing research by year in the field of cattle genomics and transcriptomics (including non-coding RNA) from January 2000 to August 2016.

Transcriptomics research by both microarray and RNA sequencing methods has allowed for a better understanding of the genes and regulatory networks of complex traits in animals [12], such as the biosynthesis of major milk components (reviewed in Refs. [13, 14]). Emerging studies now suggest that non-coding RNAs (ncRNAs) are key regulators of mammary gland development and lactation processes [15–17]. The results from the ENCODE (ENCyclopedia of DNA elements) project [18, 19] indicate that only a small portion of the genome, about

http://dx.doi.org/10.5772/67194

1.5%, codes for proteins while most of the genome is transcribed into non-coding regulatory elements or ncRNA. This indicates that ncRNAs play significant regulatory roles in complex animal traits. A similar project to functionally annotate regulatory elements in animal genomes (FAANG project, www.faang.org) started in 2014 [20] and will generate data that will foster understanding of how the genome is read and translated into complex animal traits of economic importance. Indeed, the recent explosion of data on the regulatory functions of ncRNAs proves their importance in the regulation of multiple/major biological processes impacting development, differentiation and metabolism. This chapter explores recent developments on the expression, regulation and functions of ncRNAs, in particular microRNA (miRNA) and long non-coding RNA (lncRNA), in ruminant (cattle, sheep and goat) mammary gland development and the lactation process, as well as illustrate our own studies on the roles of ncRNAs in these processes.

2. Non-coding RNAs: biosynthesis and classification

Non-coding RNAs are transcribed RNA molecules that are not translated into proteins. They play a remarkable variety of biological functions by engaging target transcripts through sequence-specific interactions. They regulate many biological processes, including gene expression (transcription, RNA processing and translation), protect genomes from foreign nucleic acids and can guide DNA synthesis or genome rearrangement [21]. In general, ncRNAs are classified according to size or function. According to size, ncRNAs are classified as (1) small or short ncRNA: <200 nucleotides in their mature forms (e.g. miRNA, PIWI-interacting RNA [piRNA], small nuclear RNA [snRNA], small nucleolar RNA [snoRNA] and endogenous small interfering RNA [siRNA]) and (2) long ncRNA: >200 nucleotides long (e.g. lncRNA). According to function, ncRNAs are classified as (1) housekeeping or translation-related ncRNAs: they are constitutively expressed and crucial for normal cellular function and viability and include tRNA, rRNA and snoRNA and (2) regulatory ncRNAs and include miRNA, lncRNA, siRNA and piRNA [22, 23]. The biogenesis of these various types of ncRNAs has been discussed extensively [23–26]. This chapter focuses particularly on the involvement of miRNA and lncRNA in ruminant mammary gland development and lactation.

2.1. MicroRNAs

MiRNAs are an abundant class of short ncRNAs of about 22 nucleotides long. They regulate a variety of cellular processes through post-transcriptional repression of gene expression. MiRNAs consequently control the activities of about 60% of all protein-coding genes and participate in the regulation of almost every cellular process investigated in mammals [25]. Mature miRNAs are generated from a series of biochemical events beginning in the nucleus and culminating in the cytoplasm [24, 27, 28]. Briefly, these events occur in several main steps as follows: (1) nuclear processing of primary miRNA transcripts (pri-miRNAs) into precursor miRNAs (pre-miRNAs) by the DiGeorge Syndrome Critical Region Gene 8 (DGCR8)/Drosha complex, (2) cytoplasmic processing of pre-miRNAs into imperfectly paired miRNA duplexes by dicer,

and (3) preferential incorporation of one strand (the 'guide' miRNA strand) onto the RNA-induced silencing complex (RISC) [25]. Most miRNA genes located in introns of protein-coding genes share the promoter of the host gene [29]. MiRNAs often have multiple transcription start sites and regulate gene expression through inhibition of translation initiation or elongation, co-translational protein degradation and premature termination of translation [25, 30].

Since the discovery of the first miRNA, lin-4, in 1993 [31] and aided by deep sequencing technologies and developments in bioinformatics processing of deep sequence data, thousands of miRNAs have been detected in humans, mouse, farm animal species and plants and deposited in the miRNA data base (**Table 1**). Due to the crucial regulatory roles of miRNAs in many biological processes across species, they are being considered as candidate biomarkers of various human diseases, such as autoimmune [32], metabolic [33] and cardiovascular diseases [34], and various types of cancers [35–37].

Species	MiRNA		lncRNA	
	Precursor	Mature	Transcripts	Genes
Cattle	808	793	22,386	23,696
Sheep	106	153	–	
Goat	267	436	–	
Pig	382	411	–	
Chicken	740	994	13,085	9681
Human	1881	2588	141,353	90,062
Mouse	1193	1915	117,405	79,940

*Data source: MiRBase release 21 (http://www.mirbase.org/[38], and NONCODE database (www.noncode.org, Noncode 2016 [39]).

Table 1. Number of detected miRNAs and lncRNAs in farm animal species, mouse and human*.

2.2. Long non-coding RNAs

Long non-coding RNAs are a diverse collection of non-coding RNAs with emerging regulatory roles in many biological processes in every branch of life [26, 40–42]. LncRNA transcripts are >200 nucleotides long and constitute the largest portion of the mammalian non-coding RNA transcriptome [40]. LncRNA closely resembles mRNA than other classes of ncRNA in terms of their biogenesis pathways and form. Most lncRNAs are transcribed by the activities of RNA polymerase II, have a 5′ terminal methylguanosine cap and are often spliced and polyadenylated [41]. Some non-polyadenylated lncRNAs arise through alternative pathways probably expressed from RNA polymerase III promoters [43, 44] or arise during splicing and small nucleolar RNA production [45]. Furthermore, some lncRNAs are regulated in different ways at different stages of their biogenesis, maturation and decay [26]. Thousands of genes encoding lncRNAs have been identified in mammalian genomes (including livestock species),

birds and plants studied so far and deposited in the NONECODE database (www.nonecode.org [39], **Table 1**).

3. MicroRNA in mammary gland development and lactation biology

3.1. Occurrence of microRNA in ruminant mammary gland and in milk

The regulatory roles of miRNAs in livestock species have emerged and are growing quickly [46, 47]. The most recent release of miRBase (release 21, http://www.mirbase.org/, [38]) contains 793 mature miRNAs for cattle, 436 for goat and 153 for sheep [38] (**Table 1**). However, with the increase in the application of RNA sequencing in expression profiling of miRNAs in different livestock species, the number of novel livestock miRNAs is expected to increase.

3.1.1. Cattle

The profiles of miRNAs in bovine MG tissue or milk have been investigated using different approaches, such as microarray [48, 49], genome sequencing [4] and RNA sequencing [50–57]. A total of 496 miRNA genes were identified following sequencing of the cattle genome of which 135 were novel [4].The expression profiles of miRNAs in MG tissues and cells facilitate discovery of novel miRNAs and also identification of candidate miRNAs for different cell types, lactation stages, periods, disease response and so on. Before the release of the bovine genome sequence, Gu et al. [49] pioneered miRNA discovery in the bovine MG by cloning and sequencing small RNAs from MG tissue followed by identification of 59 distinct bovine miRNAs. Using next-generation sequencing techniques, Chen et al. [58] identified 230 and 213 known miRNAs in cow colostrum and mature milk, respectively. The authors also observed that 108 and 8 miRNAs were upregulated and downregulated, respectively, in colostrum compared to mature milk [58]. Using microarray, Izumi et al. [59] identified 100 and 53 known miRNAs in colostrum and mature milk, respectively. Using Solexa sequencing method, Li et al. [60] reported 884 unique miRNAs sequences in the bovine MG (283 known, 505 novel and 96 conserved miRNAs). Le Guillou et al. [61] identify 167 novel miRNAs in the bovine MG, many of which were also detected in mouse MG. Analysing three milk fractions (fat, whey and cells) and mammary gland tissues, we reported 210, 200 and 249 known and 33, 31 and 36 novel miRNAs in milk fat, whey and cells, respectively, and 321 known and 176 novel miRNAs in mammary gland tissues [62]. Deep sequencing the milk fat across the lactation curve, we also identified a total of 475 known and 238 novel miRNAs [63].

3.1.2. Goat

A total of 487 miRNAs were identified when the goat genome was sequenced and the largest miRNA clusters were found on chromosome 21 [6]. Using the Illumina-Solexa high-throughput sequencing technology to analyse goat MG tissues during early lactation, Ji et al. [64]

reported 131 novel and 300 conserved miRNAs. Using the same method (Illumina-Solexa sequencing), Li et al. [65] reported 346 conserved and 95 novel miRNAs in goat MG tissues from dry off and peak lactation does.

3.1.3. Sheep

Most miRNAs identified in sheep come from tissues other than the MG. For example, Caiment et al. [66] identified 747 miRNAs from the skeletal muscle through deep sequencing, whereas McBride et al. [67] reported 212 miRNAs from sheep ovarian follicles and corpus lutea at various reproductive stages. In the MG, Galio et al. [68] showed the presence of three known miRNAs including miR-21, miR-205 and miR-200 family in pregnant and lactating sheep.

3.2. MicroRNA function in ruminant mammary gland and milk synthesis

3.2.1. Expression patterns of microRNAs in lactation stages

3.2.1.1. Temporal and spatial expression of microRNAs

Indication of involvement of miRNAs in MG functions was gained through observation of differences in type and expression levels of miRNAs between lactation stages, under different nutritional regimes and presence of disease pathogens. Li et al. [50] identified 56 miRNAs that were significantly differentially expressed between lactation and non-lactation periods. Similarly, Wang et al. [48] detected 12 downregulated miRNAs (miR-10a, miR-15b, miR-16, miR-21, miR-33b, miR-145, miR-146b, miR-155, miR-181a, miR-205, miR-221 and miR-223) in the dry period (30 days prepartum) compared to early lactation period (7 days postpartum) and one upregulated miRNA (miR-31) in early lactation compared to the dry period. Previously, we examined miRNA expression pattern during a lactation cycle to explore it regulatory mechanisms during lactation using milk fat as input tissue for sampling [63]. In a previous investigation, we have shown that milk fat miRNA transcriptome closely resemble the miRNome of MG tissue [62]. We collected samples at the lactogenesis (LAC) (day 1 and 7), galactopoiesis (GAL) (day 30, 70, 130, 170 and 230) and involution (INV) (day 290 and when milk production dropped to 5 kg/day) stages from nine cows for deep sequencing [63]. We observed that 15 miRNAs (miR-30a-5p, miR-30d, miR-21-5p, miR-26a, miR-148a, let-7a-5p, let-7b, let-7f, let-7g, miR-99a-5p, miR-191, miR-200a, miR-200c, miR-186, miR-92a) were highly expressed across lactation stages [63]. MiR-148a and miR-26a were the most abundantly expressed accounting for more than 10% of the read counts in each stage of lactation. We also performed a differential expression (DE) analysis and detected miR-29b/miR-363 and miR-874/miR-6254 as important mediators of transition signals from LAC to GAL and from GAL to INV stages, respectively [63]. Furthermore, DE analysis indicated various patterns of miRNA expression across the lactation curve. For instance, some miRNAs were highly expressed during early lactation (lactogenesis) followed by decreased expression at later stages, whereas others were slightly expressed during early lactation but showed increased expression during mid-lactation and decreased expression during late lactation and vice versa [63] (**Figure 2**).

http://dx.doi.org/10.5772/67194

(a)

miRNAs/Stages[1]	GAL_vs_LAC		INV_vs_LAC		INV_vs_GAL		Expression Pattern[2]
	Fold change	p_value	Fold change	p_value	Fold change	p_value	
miR-2285t	-2.69	1.99E-11	-2.33	7.18E-24	-6.28	9.32E-08	D_D_D
miR-744	-3.03	5.23E-13	-2.22	7.54E-03	1.74	9.75E-04	D_U_D
miR-221	-2.66	3.49E-03	4.14	8.97E-04	11.00	3.67E-12	D_U_U
miR-152	1.42	3.83E-02	-1.60	4.84E-02	-1.74	3.67E-06	U_D_D
miR-29c	6.82	2.34E-26	4.29	4.13E-10	-1.57	1.89E-02	U_D_U
miR-23a	1.28	2.55E-02	2.22	4.90E-09	1.73	2.11E-06	U_U_U

(b)

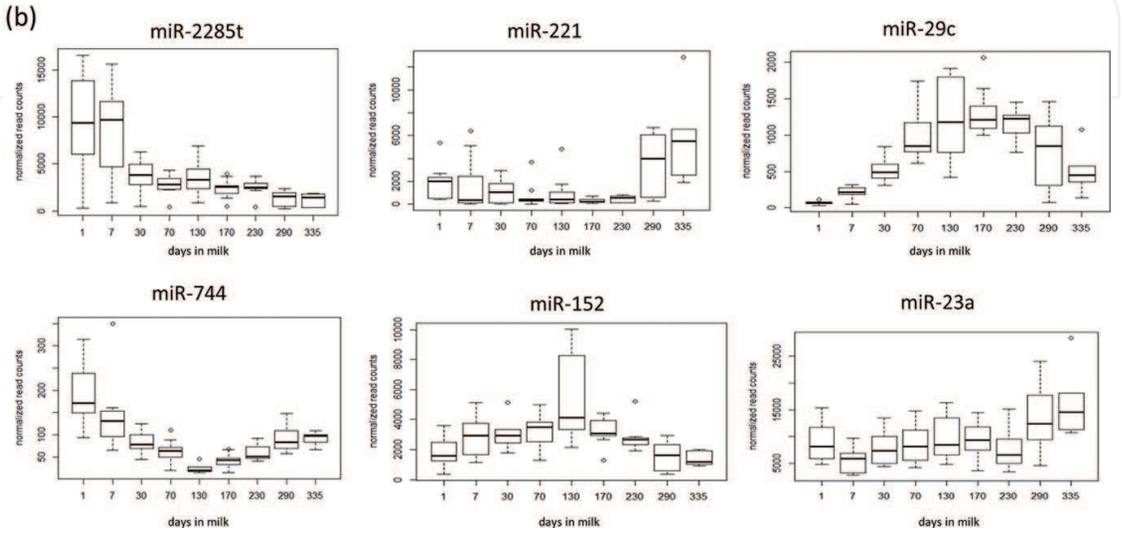

Figure 2. Differential miRNA expression patterns during a bovine lactation curve. (a) Fold change values of six miRNAs whose expression patterns changed significantly during each lactation switch and (b) box plots of their normalized read count values by lactation day. [1]LAC: lactogenesis; GAL: galactopoiesis; INV: involution; [2]D: downregulated and U: upregulated.

The temporal expression pattern of miRNAs has been reported in other ruminant species. For example, Galio et al. [68] reported a change in the expression pattern of miR-21, miR-205 and miR-200 family in MG tissues from pregnant and lactating sheep. From the early, middle and late stages of pregnancy and during lactation, the expression of miR-21 and miR-25 decreased, whereas miR-200 family (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) showed increased expression [68]. Similarly, investigating the expression pattern of miRNAs during early and peak lactation and dry period, Li et al. [65] identified 15 differentially expressed miRNAs when comparing peak lactation and dry period including three significantly highly expressed miRNAs (miR-2887, miR-451 and miR-2478) during peak lactation and 12 significantly highly expressed miRNAs (miR-199b, miR-128, miR-25, miR-145, miR-98, miR-222, miR-181b, miR-199a-3p, miR-93, miR-221, let-7b and let-7c) during the dry period.

3.2.1.2. MicroRNAs synergistically regulate lactation control mechanisms

A wealth of evidence indicates that several miRNAs can work together to regulate target genes in the same or different biological pathways [69, 70]. We have successfully characterized a group of highly interacting miRNAs (modules) using a weighted co-expression network analysis [71] and correlated important miRNA modules to milk yield and milk components [72].

We identified three consensus (BLUE [62 miRNAs], TURQUOISE [133 miRNAs] and BROWN [59 miRNAs]) modules and the GREY module reserved for unclassified genes, throughout lactation stages (**Figure 3**). Based on module trait relationship, we were able to determine important modules (with absolute correlation >0.6) for milk components at each lactation stage. The BROWN and BLUE modules were highly related to protein and somatic cell count, respectively, in early lactation, the BLUE module to somatic cells in middle lactation and the BLUE module to urea and lactose in late lactation stage. We also found the most important component or hub miRNAs, which potentially coordinated miRNA synergetic mechanisms in their respective modules. MiR-149-5b and miR-874 were hub miRNAs in the BLUE module for milk somatic cells at early and middle lactation, respectively, whereas miR-330 was the hub miRNA in the BLUE module for milk urea and lactose at late lactation (**Figure 3**). Three miRNAs (mir-149-5b, miR-874 and miR-30) in the BLUE module play important roles in cell cycle [73–77], so it could be expected that these miRNAs regulate secretion of somatic cells in milk from MG.

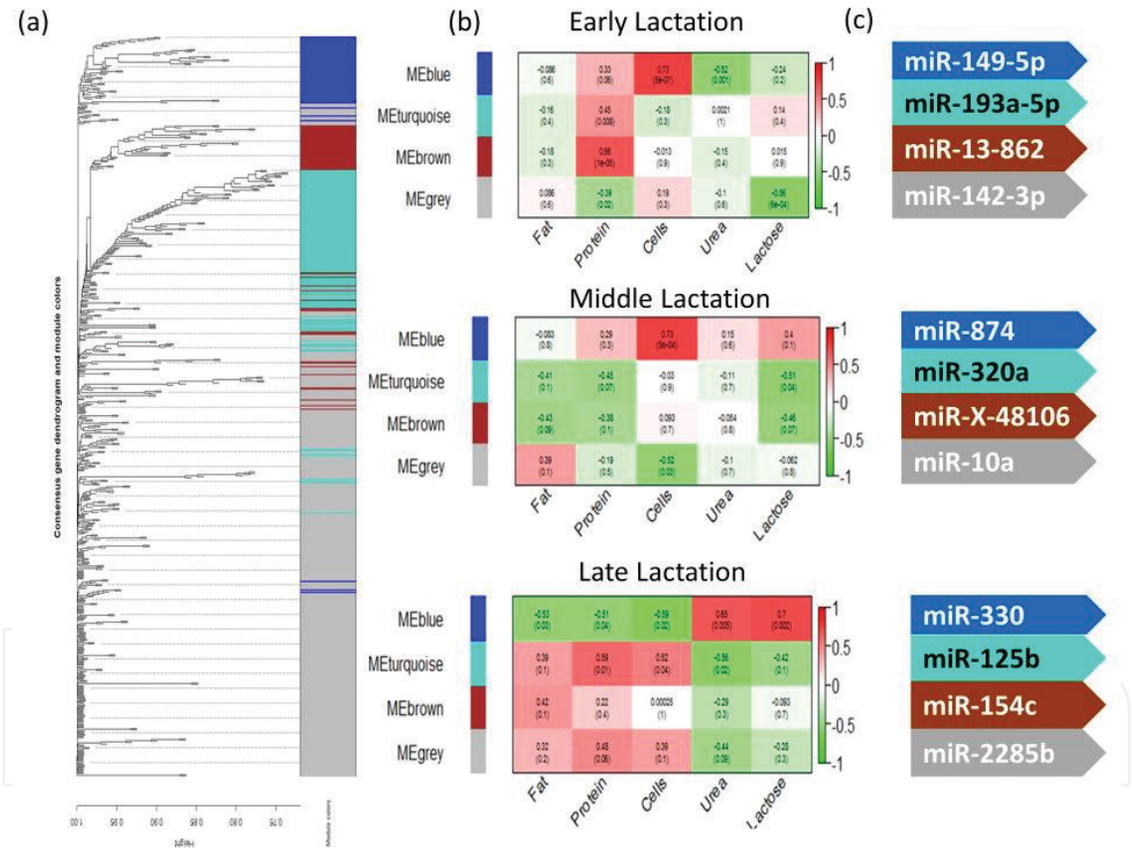

Figure 3. Important consensus modules and their hub miRNAs for milk component traits in different lactation periods. (a) Dynamic cut tree (dendrogram) based on topological overlap distance in gene expression profile; (b) module trait relationship in early, middle and late lactation and (c) hub miRNAs in the modules. GREY colour is for genes that do not belong to a specific module.

3.2.2. Networks and pathways regulated by microRNAs during a lactation cycle

Through their target genes, miRNAs have been shown to control signal transduction in different species [78]. MiRNA roles in important pathways such as transforming growth factor beta

http://dx.doi.org/10.5772/67194

(TGF-β), prolactin and protein kinase signalling in MG development and lactation have been reviewed by several authors [79–83]. MiRNA regulation of three important signalling pathways (NOTCH, PTEN and HIPPO) in MG and breast cancer cells was recently reviewed [15]. Important miRNAs regulating these pathways include mir-34, mir-29, mir-146, mir-199 and mir-200 families for NOTCH signalling pathway, miR-21 and miR-155 for PTEN signalling pathway and miR-934 for HIPPO pathway. In Canadian Holstein cows, we performed the enrichment of differentially expressed miRNA target genes to signalling pathways and noted that relevant signalling pathways for transition between lactation stages are involved in apoptosis (PTEN and SAPK/JNK), intracellular signalling (protein kinase A, TGF-β and ERK5), cell cycle regulation (STAT3), cytokines (prolactin), hormone and growth factors (growth hormone and glucocorticoid receptor). *PTEN* is an important target gene for miR-29b in the regulation of mammary gland development [84]. PTEN signalling is crucial for the activities of prolactin autocrine [85]. The initiation of lactation is known to require induction of autocrine prolactin, and the level of this autocrine is known to be endogenously regulated by the signal of PTEN-PI3K-AKT pathway [85]. **Figure 4** is an illustration of some miRNAs that target genes in relevant signalling pathways during lactation [63]. Pathways, such as PTEN and growth hormone signalling, have been identified as important for regulatory mechanisms during lactation [85, 86].

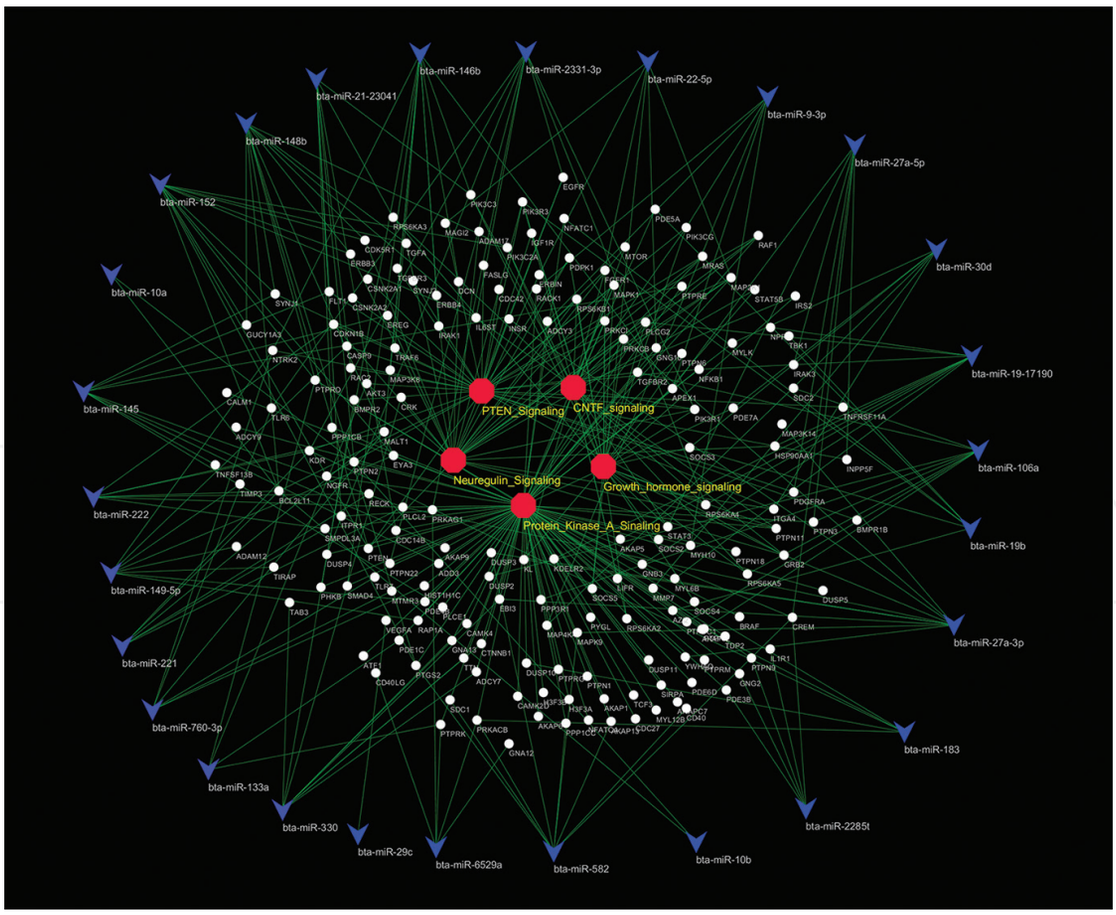

Figure 4. Illustration of miRNA-gene-pathway networks obtained from dynamic differentially expressed miRNAs during a bovine lactation curve. The outer layer shows miRNAs (blue arrow heads), which targets at least two genes (white dots) in significantly enriched pathways (red dots).

3.2.3. Functional validation of microRNA target genes

Since *in vivo* experiments for functional validation of MG miRNAs are not feasible, such studies have mostly relied on the use of knock-out/mimics and MG-specific cell types. Using bovine mammary epithelial cells (BMEC), miR-15a was shown to regulate growth hormone receptor, viability of BMEC and the expression of casein genes [86]. MiR-486 regulation of lactation by targeting the PTEN gene in cow MGs has been demonstrated [87]. Bian et al. [88] recently reported that epigenetic regulation of miR-29s affects the lactation activity of BMEC. MiR-181a was shown to regulate the biosynthesis of bovine milk fat through targeting acyl-CoA synthetase long-chain family member 1 (*ACSL1*) [89]. MiR-103 was reported to control milk fat accumulation in goat MG during lactation [90]. Moreover, miR-27a was shown to suppress triglyceride accumulation as well as altered gene expression associated with fat metabolism in dairy goat mammary epithelial cells (GMEC) [91]. In another study, miR-135a was reported to target and regulate prolactin receptor (*PRLR*) gene in GMEC [92]. Inhibition of the expression of miR-145 in GMEC was shown to increase methylation levels of fatty acid synthase (*FASN*), stearoyl-CoA desaturase 1 (*SCD1*), peroxisome proliferator-activated receptor gamma (*PPARG*) and sterol regulatory element binding transcription factor 1 (*SREBF1*) [93]. MiR-24 control of triacylglycerol synthesis in goat mammary epithelial cells by targeting *FASN* gene has been demonstrated [94]. The ability of miR-145 to regulate lipogenesis in GMEC through targeting insulin-induced gene 1 (*INSIG1*) and epigenetic regulation of lipid-related genes has been demonstrated [93]. MiR-143 was shown to inhibit proliferation as well as induce apoptosis of GMEC [95]. MiR130b regulation of PPARγ coactivator-1α suppressed fat metabolism in GMEC [96]. In non-ruminant species, many miRNAs, including let-7 family members, mir-17/92, miR-30b, miR-93, miR-99a and miR-b, miR-101a, miR-126-3p, miR-138, miR-146b, miR-200 family members, mir-203, miR-205, miR-206, miR-210, miR-212/132, miR-221 and miR-424/50, have been reported to play roles in mammary gland development and disease [15]. Some miRNAs with functionally validated targets are summarized in **Table 2**.

3.3. Nutritional modulation of microRNA expression and function

The miRNA expression profile in response to dietary treatments has been studied in adipose tissues of lambs and cattle and bovine mammary gland tissues [56, 100–102]. A change in diet that interferes with energy balance has been shown to change miRNA expression pattern in cow liver [103]. Wang et al. [104] fed cows with high- and low-quality forage diets (corn stover and rice straw) and showed that miR-125b, miR-141, miR-181a, miR-221 and miR-15b changed their expression patterns across different tissues including MG. We have examined the expression pattern of miRNAs following MG adaptation to dietary supplementation with 5% linseed oil or 5% safflower oil using miRNA sequencing and identified seven differentially regulated miRNAs, including six upregulated (miR-199c, miR-199a-3p, miR-98, miR-378, miR-148b and miR-21-5p) and one downregulated (miR-200a) by both linseed and safflower oil. The target genes of these seven miRNAs have functions related to gene expression and general cellular metabolism and are enriched in four pathways of lipid metabolism (3-phosphoinositide biosynthesis, 3-phosphoinositide degradation, D-myo-inisitol-5-phosphate metabolism and the superpathway of inositol phosphate compounds) [51]. The largest number of target genes

(39) were associated with two functions (synthesis of lipid and concentration of lipid) related with lipogenesis. In goat, Mobuchon et al. [105] detected 30 miRNAs with expression patterns potentially modulated by food deprivation (14 and 16 were upregulated and downregulated, respectively). Among them, miR-204-5p and miR-223-3p were most remarkably affected by food deprivation and potentially played roles in the nutritional regulation of gene expression in the MG.

MiRNAs	Target genes	Main consequence	Cell	References
miR-181	*ACSL1*	Decrease lipid synthesis	BMEC	[89]
miR-29 family	*DNMT3A DNMT3B*	Decrease global DNA methylation	BMEC	[88]
miR-152	*DNMT1*	Decrease global DNA methylation and increase expression of Akt and PPARγ	BMEC	[97]
miR-486	*PTEN*	Alter expression of downstream genes of PTEN (AKT, mTOR pathways)	BMEC	[87]
miR-181b	*IRS2*	Wnt signalling pathway in GMEC	GMEC	[98]
miR-27a	*PPARγ*	Decrease triglyceride accumulation	GMEC	[91]
miR-26a and b	*INSIG1*	Decrease triacylglycerol synthesis	GMEC	[99]
miR-24	*FASN, SREBF1, ACACA*	Decrease triacylglycerol synthesis	GMEC	[94]
miR-15a	*GHR*	Inhibit viability of mammary epithelial cells	BMEC	[86]
miR-130b	*PPARGC1A*	Repress PPARGC1A expression	GMEC	[96]
miR-143	*BAX and BCL-2*	Inhibit proliferation and induce apoptosis	GMEC	[95]
miR145	*INSIG1*	Increase fat droplet formation, triacylglycerol accumulation and proportion of unsaturated fatty acids	GMEC	[93]

Table 2. MicroRNAs with functionally validated target genes using ruminant mammary gland cells.

3.4. MicroRNA functions in mammary gland health

MiRNAs have been shown to play roles in bovine infection and immunity in a wide range of tissues [54, 106–113]. For mammary gland, Naeem et al. [114] studied the expression of 14 miRNAs (miR-10a, miR-15b, miR-16a, miR-17, miR-21, miR-31, miR-145, miR-146a, miR-146b, miR-155, miR-181a, miR-205, miR-221 and miR-223) in MG tissue challenged with *Streptococcus uberis* and identified three downregulated miRNAs (miR-181a, miR-16 and miR-31) and one upregulated miRNA (miR-223) in infected versus healthy tissue. Lawless et al. [107] showed that 21 miRNAs were differentially expressed upon *Streptococcus uberis* infection of bovine primary epithelial cells. Using BMEC, Jin et al. [108] reported a differential expression of nine miRNAs (miR-184, miR-24-3p, miR-148, miR-486, let-7a-5p, miR-2339, miR-499, miR-23a and miR-99b) upon challenge with heat inactivated *Escherichia coli* and *Staphylococcus aureus* bacteria. Hou et al. [115] identified three upregulated miRNAs (miR-296, miR-2430 and miR-671) and one downregulated miRNA (miR-2318) in mastitis affected compared with healthy mammary gland tissues. Li et al. [111] sequenced RNA isolated

from *S. aureus*-induced mastitis and control cows and identified 77 miRNAs with significant expression differences between the two groups. Li et al. [116] showed that miR-23 might be an important immune miRNA through its target mastitis candidate gene, high mobility group box 1 *(HMGB1)*.

3.5. MicroRNA function in milk recipients

Recent evidence suggesting that milk-derived miRNAs may have potential regulatory roles in modulating the immune system or metabolic processes of milk recipients still remain controversial [117–124]. Currently, there are two hypotheses about miRNA function in infants/offspring: the first proposes that milk miRNAs exert physiological regulatory functions after transferring to offspring, and the second assumes that miRNAs do not have any function but merely provide nutrition. According to Zhang et al. [117], the rice-derived miRNA, miR-168a, can bind to the mRNA of human/mouse low-density lipoprotein receptor adapter protein 1 (*LDLRAP1*) and inhibit its expression in the liver, and consequently decrease LDL removal from mouse plasma. Baier et al. [118] reported that miR-29b-3p and miR-200c-3p could be absorbed by humans in biologically meaningful amounts, which could affect related gene expression in peripheral blood mononuclear cells while Izumi et al. [125] confirmed that whey exosomes containing miRNAs and mRNA could be absorbed by human macrophages. These results opened a new aspect of the nutritional control of metabolism [119]. However, other studies have not succeeded to validate the hypothesis that milk miRNAs exert physiological regulatory functions after transferring to offspring [126–129]. For instance, Auerbach et al. [129] observed that drinking bovine milk increased circulating levels of miRNAs (miR-29b-3p and miR-200c-3p) but found no evidence that they significantly altered miRNA signals after milk ingestion. These authors concluded that milk miRNAs likely serve as a source of nutrition but not as post-transcriptional regulators in recipients.

4. Long non-coding RNA in mammary gland development and lactation biology

4.1. Prolife and expression of long non-coding RNAs

A limited number of studies have examined the occurrence and potential functions of lncRNAs in ruminant livestock species [130–132]. A pioneer study screened reconstructed transcript assemblies of bovine-specific expressed sequence tags and identified 449 putative lncRNAs located in 405 intergenic regions [130]. Following this initial study, Weikard et al. [131] used RNA sequencing technique and identified 4848 potential lncRNAs, which were predominantly intergenic (4365) in bovine skin. In another study, Billerey et al. [132] characterized 584 lncRNAs in bovine muscle in addition to significant correlated expression between 2083 pairs of lncRNA/protein encoding genes. Koufariotis et al. [133] characterized the lncRNA repertoire across 18 bovine tissues including the mammary gland and reported 9778 transcripts. Ibeagha-Awemu et al. [134] studied the lncRNA profile of the

bovine mammary gland by RNA sequencing and identified 4227 lncRNAs (338 known and 3889 novel). In goats, Zhan et al. [135] sequenced libraries from developing longissimus dorsi fetal (45, 60 and 105 days of gestation) and postnatal (3 days after birth) muscles and identified 3981 lncRNA transcripts corresponding to 2739 lncRNA genes. Ren et al. [136] identified 1336 specific lncRNAs in fetal skin of Youzhou dark goat (dark skin) and Yudong white goat (white skin). Similarly, Chao et al. [137] in a study with aim to identify and classify new transcripts in Dorper and small-tail Han sheep muscle transcriptomes predicted with high confidence 1520 transcripts to be lncRNAs.

4.2. Function of long non-coding RNAs

While the regulatory roles of lncRNAs have been associated with several human disease conditions including tumourigenesis, cardiac development, aging and immune system development [138–143], little information exist on livestock species. Our previous study on bovine mammary gland identified 26 lncRNAs that were significantly differentially regulated in response to a diet rich in α-linolenic acid thus suggesting potential regulatory roles of lncRNAs in fatty acid synthesis and lipid metabolism [134]. In a study with goat fetal muscle tissues at different stages of development, Zhan et al. [135] identified 577 significantly differentially expressed lncRNA transcripts thus suggesting roles in muscle development.

5. Genome editing technology and non-coding RNA

Genome engineering has been considered as the next genomic revolution [144], and it is expected to significantly improve livestock production by precision genome editing [145–147] favouring markers associated with improved productivity, reproduction and health status. The history of genome editing in livestock has been extensively reviewed [145, 148–150]. The advent of engineered endonucleases (EENs), including zinc finger nucleases (ZFNs) [151], transcription activator-like effector nucleases (TALENs) [152] and clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) [153]), allows to cut a specific position in DNA sequence and then use endogenous cellular pathways to direct DNA repair to introduce specified alterations to the DNA sequence. Genome-editing approaches have been successfully used in different livestock species, such as pig [154, 155], goat [156], cattle [157] and sheep [158]. In dairy cows, these technologies have been used to manipulate the genome so that they produce specific milk types, such as milk that causes less allergic problems (e.g. milk with less β-lactoglobulin protein) [159, 160]. These genome-editing tools also helped to improve mammary gland health by generating mastitis-resistant cattle [161, 162]. From an animal breeding perspective, a simulation study showed that genomic prediction combined with genome editing could be of benefit [163]. A total of 10,000 additive loci were simulated and shown to contribute to the variation in selected traits and benefits could be achieved with only 20 of those loci being edited in each selected sire [163]. Similar to other genome sequences, miRNA gene sequences within mammalian genomes can be easily edited with high efficacy and precision [144]. Targeted miRNA editing will enable revelation of the

complex regulatory circuits governed by miRNAs and realization, in the long term, of their full diagnostic and therapeutic potentials. For instance, Chen et al. [164] successfully used TALEN to disrupt the function of miR-21 in cancerous cells. A transgenic calf engineered to express miRNA-4 and miR-6 showed an absence of β-lactoglobulin and a concurrent increase in casein proteins in milk [165].

6. Conclusion and remarks

Up to now, it is well known that the mammalian genome encodes thousands of ncRNAs and these ncRNAs play important roles in many processes related to MG development, health and disease as well as roles in milk secretion and lactation processes. Regarding animal breeding, several ncRNAs target specific processes and their target genes could be important biomarkers for specific traits of interest. Therefore, the application of ncRNA to improve mammary gland health and milk production as well as enhance milk quality is very promising. However, the first step is a better understanding of ncRNA function in MG development and lactation. In fact, the MG is a complex tissue and lactation is a complicated process, but what we known about the regulatory networks underling MG function and the lactation process is very limited. For instance, through RNA sequencing, many novel ncRNAs have been detected in the MG but knowledge of their actual functions remains elusive. Therefore, integrated 'omics' approaches (genomics, transcriptomics, epigenomics and proteomics) should be used to identify and explore the potential roles of ncRNAs in mammary gland development and lactation biology. Moreover, a miRNA can target hundreds of genes thus making it difficult, costly and labour-intensive to functionally validate each miRNA gene target. Thus, integrative approaches such as combination of miRNA and mRNA expression in the same sample will refine computational predictions and increase our understanding of miRNA function and its application.

Acknowledgements

We acknowledge financial support from Agriculture and Agri-Food Canada.

Author details

Duy N. Do[1,2] and Eveline M. Ibeagha-Awemu[1]*

*Address all correspondence to: Eveline.ibeagha-awemu@agr.gc.ca

1 Agriculture and Agri-Food Canada, Sherbrooke Research and Development Centre, Sherbrooke, Quebec, Canada

2 Department of Animal Science, McGill University, Ste-Anne-De-Bellevue, Quebec, Canada

References

[1] Lemay DG, Neville MC, Rudolph MC, Pollard KS, German JB: Gene regulatory networks in lactation: Identification of global principles using bioinformatics. BMC Systems Biology 2007, **1**(1):1-24.

[2] Strucken EM, Laurenson YCSM, Brockmann GA: Go with the flow—Biology and genetics of the lactation cycle. Frontiers in Genetics 2015, 6: 118 (11 pages).

[3] Zimin AV, Delcher AL, Florea L, Kelley DR, Schatz MC, Puiu D, Hanrahan F, Pertea G, Van Tassell CP, Sonstegard TS: A whole-genome assembly of the domestic cow, Bos taurus. Genome Biology 2009, **10**(4):1.

[4] Elsik CG, Tellam RL, Worley KC: The genome sequence of taurine cattle:a window to ruminant biology and evolution. Science 2009, **324**(5926):522-528.

[5] Jiang Y, Xie M, Chen W, Talbot R, Maddox JF, Faraut T, Wu C, Muzny DM, Li Y, Zhang W: The sheep genome illuminates biology of the rumen and lipid metabolism. Science 2014, **344**(6188):1168-1173.

[6] Dong Y, Xie M, Jiang Y, Xiao N, Du X, Zhang W, Tosser-Klopp G, Wang J, Yang S, Liang J: Sequencing and automated whole-genome optical mapping of the genome of a domestic goat (Capra hircus). Nature Biotechnology 2013, **31**(2):135-141.

[7] Matukumalli LK, Lawley CT, Schnabel RD, Taylor JF, Allan MF, Heaton MP, O'Connell J, Moore SS, Smith TP, Sonstegard TS: Development and characterization of a high density SNP genotyping assay for cattle. PLoS One 2009, **4**(4):e5350.

[8] Boichard D, Chung H, Dassonneville R, David X, Eggen A, Fritz S, Gietzen KJ, Hayes BJ, Lawley CT, Sonstegard TS: Design of a bovine low-density SNP array optimized for imputation. PLoS One 2012, **7**(3):e34130.

[9] Tosser-Klopp G, Bardou P, Bouchez O, Cabau C, Crooijmans R, Dong Y, Donnadieu-Tonon C, Eggen A, Heuven HC, Jamli S: Design and characterization of a 52K SNP chip for goats. PLoS One 2014, **9**(1):e86227.

[10] Archibald A, Cockett N, Dalrymple B, Faraut T, Kijas J, Maddox J, McEwan J, Hutton Oddy V, Raadsma H, Wade C: The sheep genome reference sequence: A work in progress. Animal Genetics 2010, **41**(5):449-453.

[11] Kijas JW, Townley D, Dalrymple BP, Heaton MP, Maddox JF, McGrath A, Wilson P, Ingersoll RG, McCulloch R, McWilliam S: A genome wide survey of SNP variation reveals the genetic structure of sheep breeds. PloS One 2009, **4**(3):e4668.

[12] Wickramasinghe S, Cánovas A, Rincón G, Medrano JF: RNA-sequencing: A tool to explore new frontiers in animal genetics. Livestock Science 2014, **166**:206-216.

[13] Osorio JS, Lohakare J, Bionaz M: Biosynthesis of milk fat, protein, and lactose: Roles of transcriptional and posttranscriptional regulation. Physiological Genomics 2016, **48**(4):231-256.

[14] Bionaz M, Loor J, Hurley W: Milk protein synthesis in the lactating mammary gland: Insights from transcriptomics analyses. INTECH Open Access Publisher, Rijeka, Croatia; 2012.

[15] Sandhu GK, Milevskiy MJ, Wilson W, Shewan AM, Brown MA: Non-coding RNAs in Mammary Gland Development and Disease. Advances in Experimental Medicine and Biology, Berlin, Germany, 2016, 886:121-153.

[16] Standaert L, Adriaens C, Radaelli E, Van Keymeulen A, Blanpain C, Hirose T, Nakagawa S, Marine J-C: The long noncoding RNA Neat1 is required for mammary gland development and lactation. RNA 2014, **20**(12):1844-1849.

[17] Hansji H, Leung EY, Baguley BC, Finlay GJ, Askarian-Amiri ME: Keeping abreast with long non-coding RNAs in mammary gland development and breast cancer. Frontiers in Genetics 2014, **5**:379.

[18] Birney E, Stamatoyannopoulos JA, Dutta A, Guigó R, Gingeras TR, Margulies EH, Weng Z, Snyder M, Dermitzakis ET, Thurman RE: Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature 2007, **447**(7146):799-816.

[19] ENCODE_Project_Consortium: An integrated encyclopedia of DNA elements in the human genome. Nature 2012, **489**(7414):57-74.

[20] Andersson L, Archibald AL, Bottema CD, Brauning R, Burgess SC, Burt DW, Casas E, Cheng HH, Clarke L, Couldrey C: Coordinated international action to accelerate genome-to-phenome with FAANG, the Functional Annotation of Animal Genomes project. Genome Biology 2015, **16**(1):1.

[21] Cech TR, Steitz JA: The noncoding RNA revolution—trashing old rules to forge new ones. Cell 2014, **157**(1):77-94.

[22] Santosh B, Varshney A, Yadava PK: Non-coding RNAs: Biological functions and applications. Cell Biochemistry and Function 2015, **33**(1):14-22.

[23] Morris KV, Mattick JS: The rise of regulatory RNA. Nature Reviews Genetics 2014, **15**(6):423.

[24] Kim VN, Han J, Siomi MC: Biogenesis of small RNAs in animals. Nature Reviews Molecular Cell Biology 2009, **10**(2):126-139.

[25] Bartel DP: MicroRNAs. Cell 2004, **116**(2):281-297.

[26] Quinn JJ, Chang HY: Unique features of long non-coding RNA biogenesis and function. Nature Reviews Genetics 2016, **17**(1):47-62.

[27] Krol J, Loedige I, Filipowicz W: The widespread regulation of microRNA biogenesis, function and decay. Nature Reviews Genetics 2010, **11**(9):597-610.

[28] Filipowicz W, Bhattacharyya SN, Sonenberg N: Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nature Reviews Genetics 2008, **9**(2):102-114.

[29] Borchert GM, Lanier W, Davidson BL: RNA polymerase III transcribes human microRNAs. Nature Structural & Molecular Biology 2006, **13**(12):1097-1101.

[30] Brodersen P, Voinnet O: Revisiting the principles of microRNA target recognition and mode of action. Nature reviews Molecular Cell Biology 2009, **10**(2):141-148.

[31] Lee RC, Feinbaum RL, Ambros V: The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993, **75**(5):843-854.

[32] Pauley KM, Cha S, Chan EK: MicroRNA in autoimmunity and autoimmune diseases. Journal of Autoimmunity 2009, **32**(3):189-194.

[33] Rottiers V, Näär AM: MicroRNAs in metabolism and metabolic disorders. Nature Reviews Molecular Cell Biology 2012, **13**(4):239-250.

[34] Thum T: MicroRNA therapeutics in cardiovascular medicine. EMBO Molecular Medicine 2012, **4**(1):3-14.

[35] Alvarez-Garcia I, Miska EA: MicroRNA functions in animal development and human disease. Development 2005, **132**(21):4653-4662.

[36] Calin GA, Croce CM: MicroRNA signatures in human cancers. Nature Reviews Cancer 2006, **6**(11):857-866.

[37] Esquela-Kerscher A, Slack FJ: Oncomirs—microRNAs with a role in cancer. Nature Reviews Cancer 2006, **6**(4):259-269.

[38] Kozomara A, Griffiths-Jones S: miRBase: Annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Research 2014, **42**(D1):D68-D73.

[39] Zhao Y, Li H, Fang S, Kang Y, Hao Y, Li Z, Bu D, Sun N, Zhang MQ, Chen R: NONCODE 2016: An informative and valuable data source of long non-coding RNAs. Nucleic Acids Research 2015:gkv1252.

[40] Mercer TR, Dinger ME, Mattick JS: Long non-coding RNAs: Insights into functions. Nature Reviews Genetics 2009, **10**(3):155-159.

[41] Mercer TR, Mattick JS: Structure and function of long noncoding RNAs in epigenetic regulation. Nature Structural & Molecular Biology 2013, **20**(3):300-307.

[42] Vance KW, Ponting CP: Transcriptional regulatory functions of nuclear long noncoding RNAs. Trends in Genetics 2014, **30**(8):348-355.

[43] Dieci G, Fiorino G, Castelnuovo M, Teichmann M, Pagano A: The expanding RNA polymerase III transcriptome. Trends in Genetics 2007, **23**(12):614-622.

[44] Kapranov P, Cheng J, Dike S, Nix DA, Duttagupta R, Willingham AT, Stadler PF, Hertel J, Hackermüller J, Hofacker IL: RNA maps reveal new RNA classes and a possible function for pervasive transcription. Science 2007, **316**(5830):1484-1488.

[45] Yin Q-F, Yang L, Zhang Y, Xiang J-F, Wu Y-W, Carmichael GG, Chen L-L: Long noncoding RNAs with snoRNA ends. Molecular Cell 2012, **48**(2):219-230.

[46] Wang X, Gu Z, Jiang H: MicroRNAs in farm animals. Animal 2013, **7**(10):1567-1575.

[47] Fatima A, Morris DG: MicroRNAs in domestic livestock. Physiological Genomics 2013, **45**(16):685-696.

[48] Wang M, Moisá S, Khan M, Wang J, Bu D, Loor J: MicroRNA expression patterns in the bovine mammary gland are affected by stage of lactation. Journal of Dairy Science 2012, **95**(11):6529-6535.

[49] Gu Z, Eleswarapu S, Jiang H: Identification and characterization of microRNAs from the bovine adipose tissue and mammary gland. FEBS Letters 2007, **581**(5):981-988.

[50] Li Z, Liu H, Jin X, Lo L, Liu J: Expression profiles of microRNAs from lactating and non-lactating bovine mammary glands and identification of miRNA related to lactation. BMC Genomics 2012, **13**(1):1.

[51] Wicik Z, Gajewska M, Majewska A, Walkiewicz D, Osińska E, Motyl T: Characterization of microRNA profile in mammary tissue of dairy and beef breed heifers. Journal of Animal Breeding and Genetics 2016, **133**(1):31-42.

[52] Wang D, Liang G, Wang B, Sun H, Liu J, Guan LL: Systematic microRNAome profiling reveals the roles of microRNAs in milk protein metabolism and quality: Insights on low-quality forage utilization. Scientific Reports 2016, **6**:21194.

[53] Li R, Dudemaine P-L, Zhao X, Lei C, Ibeagha-Awemu EM: Comparative analysis of the miRNome of bovine milk fat, whey and cells. PLoS One 2016, **11**(4):e0154129.

[54] Sun J, Aswath K, Schroeder SG, Lippolis JD, Reinhardt TA, Sonstegard TS: MicroRNA expression profiles of bovine milk exosomes in response to Staphylococcus aureus infection. BMC Genomics 2015, **16**(1):806.

[55] Li R, Zhang C-L, Liao X-X, Chen D, Wang W-Q, Zhu Y-H, Geng X-H, Ji D-J, Mao Y-J, Gong Y-C et al: Transcriptome microRNA profiling of bovine mammary glands infected with Staphylococcus aureus. International Journal of Molecular Sciences 2015, **16**(3):4997-5013.

[56] Li R, Beaudoin F, Ammah AA, Bissonnette N, Benchaar C, Zhao X, Lei C, Ibeagha-Awemu EM: Deep sequencing shows microRNA involvement in bovine mammary gland adaptation to diets supplemented with linseed oil or safflower oil. BMC Genomics 2015, **16**(1):1-16.

[57] Jin W, Ibeagha-Awemu EM, Liang G, Beaudoin F, Zhao X, Guan L: Transcriptome microRNA profiling of bovine mammary epithelial cells challenged with Escherichia

coli or Staphylococcus aureus bacteria reveals pathogen directed microRNA expression profiles. BMC Genomics 2014, 15:181 (16 pages).

[58] Chen X, Gao C, Li H, Huang L, Sun Q, Dong Y, Tian C, Gao S, Dong H, Guan D: Identification and characterization of microRNAs in raw milk during different periods of lactation, commercial fluid, and powdered milk products. Cell Research 2010, **20**(10):1128-1137.

[59] Izumi H, Kosaka N, Shimizu T, Sekine K, Ochiya T, Takase M: Bovine milk contains microRNA and messenger RNA that are stable under degradative conditions. Journal of Dairy Science 2012, **95**(9):4831-4841.

[60] Li Z, Liu H, Jin X, Lo L, Liu J: Expression profiles of microRNAs from lactating and non-lactating bovine mammary glands and identification of miRNA related to lactation. BMC Genomics 2012, **13**:731-731.

[61] Le Guillou S, Marthey S, Laloë D, Laubier J, Mobuchon L, Leroux C, Le Provost F: Characterisation and comparison of lactating mouse and bovine mammary gland miRNomes. PloS One 2014, **9**(3):e91938.

[62] Li R, Dudemaine PL, Zhao X, Lei C, Ibeagha-Awemu EM: Comparative analysis of the miRNome of bovine milk fat, whey and cells. PLoS One 2016, **11**.

[63] Do DN, Li R, Dudemaine P-L, Ibeagha-Awemu EM: MicroRNA roles in signaling during lactation: An insight from differential expression, time course and pathway analyses of deep sequence data. Scientific Reports 2017 (Revised version submitted).

[64] Ji Z, Wang G, Xie Z, Zhang C, Wang J: Identification and characterization of microRNA in the dairy goat (Capra hircus) mammary gland by Solexa deep-sequencing technology. Molecular Biology Reports 2012, **39**(10):9361-9371.

[65] Li Z, Lan X, Guo W, Sun J, Huang Y, Wang J, Huang T, Lei C, Fang X, Chen H: Comparative transcriptome profiling of dairy goat microRNAs from dry period and peak lactation mammary gland tissues. PLoS One 2012, **7**(12):e52388.

[66] Caiment F, Charlier C, Hadfield T, Cockett N, Georges M, Baurain D: Assessing the effect of the CLPG mutation on the microRNA catalog of skeletal muscle using high-throughput sequencing. Genome Research 2010, **20**(12):1651-1662.

[67] McBride D, Carre W, Sontakke S, Hogg CO, Law A, Donadeu FX, Clinton M: Identification of miRNAs associated with the follicular–luteal transition in the ruminant ovary. Reproduction 2012, **144**(2):221-233.

[68] Galio L, Droineau S, Yeboah P, Boudiaf H, Bouet S, Truchet S, Devinoy E: MicroRNA in the ovine mammary gland during early pregnancy: spatial and temporal expression of miR-21, miR-205, and miR-200. Physiological Genomics 2013, **45**(4):151-161.

[69] Yepes S, López R, Andrade RE, Rodriguez-Urrego PA, López-Kleine L, Torres MM: Co-expressed miRNAs in gastric adenocarcinoma. Genomics 2016, **108**(2):93-101.

[70] Wu J, Gong H, Bai Y, Zhang W: Analyzing the miRNA-gene networks to mine the important miRNAs under skin of human and mouse. BioMed Research International 2016: 5469371 (9 pages).

[71] Langfelder P, Horvath S: WGCNA: An R package for weighted correlation network analysis. BMC Bioinformatics 2008, 9:559 (13 pages).

[72] Ibeagha-Awemu E.M: MicroRNAs are master regulators of the bovine lactation curve. Invited presentation at Plant and Animal Genome XXIV Conference (Animal Epigenetics Workshop), January 9-13, 2016, San Diego, CA, USA

[73] Lee K-H, Chen Y-L, Yeh S-D, Hsiao M, Lin J, Goan Y, Lu P-J: MicroRNA-330 acts as tumor suppressor and induces apoptosis of prostate cancer cells through E2F1-mediated suppression of Akt phosphorylation. Oncogene 2009, **28**(38):3360-3370.

[74] Li Y, Zhu X, Xu W, Wang D, Yan J: miR-330 regulates the proliferation of colorectal cancer cells by targeting Cdc42. Biochemical and Biophysical Research Communications 2013, **431**(3):560-565.

[75] Wang L, Gao W, Hu F, Xu Z, Wang F: MicroRNA-874 inhibits cell proliferation and induces apoptosis in human breast cancer by targeting CDK9. FEBS Letters 2014, **588**(24):4527-4535.

[76] Lin RJ, Lin YC, Yu AL: miR-149* induces apoptosis by inhibiting Akt1 and E2F1 in human cancer cells. Molecular Carcinogenesis 2010, **49**(8):719-727.

[77] Pan S, Zhan S, Pei B, Sun Q, Bian L, Sun B: MicroRNA-149 inhibits proliferation and invasion of glioma cells via blockade of AKT1 signaling. International Journal of Immunopathology and Pharmacology 2012, **25**(4):871-881.

[78] Inui M, Martello G, Piccolo S: MicroRNA control of signal transduction. Nature Reviews Molecular Cell Biology 2010, **11**(4):252-263.

[79] Hennighausen L, Robinson GW, Wagner K-U, Liu X: Prolactin signaling in mammary gland development. Journal of Biological Chemistry 1997, **272**(12):7567-7569.

[80] Hennighausen L, Robinson GW: Signaling pathways in mammary gland development. Developmental Cell 2001, **1**(4):467-475.

[81] Hennighausen L, Robinson GW: Information networks in the mammary gland. Nature Reviews Molecular Cell Biology 2005, **6**(9):715-725.

[82] Callahan R, Raafat A: Notch signaling in mammary gland tumorigenesis. Journal of Mammary Gland Biology and Neoplasia 2001, **6**(1):23-36.

[83] Wakefield LM, Piek E, Böttinger EP: TGF-β signaling in mammary gland development and tumorigenesis. Journal of Mammary Gland Biology and Neoplasia 2001, **6**(1):67-82.

[84] Wang Z, Hou X, Qu B, Wang J, Gao X, Li Q: Pten regulates development and lactation in the mammary glands of dairy cows. PLoS One 2014, **9**(7):e102118.

[85] Chen C-C, Stairs DB, Boxer RB, Belka GK, Horseman ND, Alvarez JV, Chodosh LA: Autocrine prolactin induced by the Pten–Akt pathway is required for lactation initiation and provides a direct link between the Akt and Stat5 pathways. Genes & Development 2012, **26**(19):2154-2168.

[86] Li H-M, Wang C-M, Li Q-Z, Gao X-J: MiR-15a decreases bovine mammary epithelial cell viability and lactation and regulates growth hormone receptor expression. Molecules 2012, **17**(10):12037-12048.

[87] Li D, Xie X, Wang J, Bian Y, Li Q, Gao X, Wang C: MiR-486 regulates lactation and targets the PTEN gene in cow mammary glands. PLoS One 2015, **10**(3):e0118284.

[88] Bian Y, Lei Y, Wang C, Wang J, Wang L, Liu L, Liu L, Gao X, Li Q: Epigenetic regulation of miR-29s affects the lactation activity of dairy cow mammary epithelial cells. Journal of Cellular Physiology 2015, **230**(9):2152-2163.

[89] Lian S, Guo J, Nan X, Ma L, Loor J, Bu D: MicroRNA Bta-miR-181a regulates the biosynthesis of bovine milk fat by targeting ACSL1. Journal of Dairy Science 2016, **99**(5):3916-3924.

[90] Lin X, Luo J, Zhang L, Wang W, Gou D: MiR-103 controls milk fat accumulation in goat (Capra hircus) mammary gland during lactation. PLoS One 2013, **8**(11):e79258.

[91] Lin X-z, Luo J, Zhang L-p, Wang W, Shi H-b, Zhu J-j: MiR-27a suppresses triglyceride accumulation and affects gene mRNA expression associated with fat metabolism in dairy goat mammary gland epithelial cells. Gene 2013, **521**(1):15-23.

[92] Ji Z, Dong F, Wang G, Hou L, Liu Z, Chao T, Wang J: miR-135a targets and regulates prolactin receptor gene in goat mammary epithelial cells. DNA and Cell Biology 2015, **34**(8):534-540.

[93] Wang H, Shi H, Luo J, Yi Y, Yao D, Zhang X, Ma G, Loor JJ: MiR-145 regulates lipogenesis in goat mammary cells via targeting INSIG1 and epigenetic regulation of lipid-related genes. Journal of Cellular Physiology 2016, 9999: 1–11.

[94] Wang H, Luo J, Chen Z, Cao W, Xu H, Gou D, Zhu J: MicroRNA-24 can control triacylglycerol synthesis in goat mammary epithelial cells by targeting the fatty acid synthase gene. Journal of Dairy Science 2015, **98**(12):9001-9014.

[95] Ji Z, Wang G, Hou L, Liu Z, Wang J, Chao T: miR-143 inhibits proliferation and induces apoptosis of mammary epithelial cells in dairy goat. Animal Cells and Systems 2016, **20**(2):63-69.

[96] Chen Z, Luo J, Ma L, Wang H, Cao W, Xu H, Zhu J, Sun Y, Li J, Yao D: MiR130b-regulation of PPARγ coactivator-1α suppresses fat metabolism in goat mammary epithelial cells. PLoS One 2015, **10**(11):e0142809.

[97] Wang J, Bian Y, Wang Z, Li D, Wang C, Li Q, Gao X: MicroRNA-152 regulates DNA methyltransferase 1 and is involved in the development and lactation of mammary glands in dairy cows. PLoS One 2014, **9**(7):e101358.

[98] Lian S, Guo JR, Nan XM, Ma L, Loor JJ, Bu DP: MicroRNA Bta-miR-181a regulates the biosynthesis of bovine milk fat by targeting ACSL1. Journal of Dairy Science 2016, **99**(5):3916-3924.

[99] Wang H, Luo J, Zhang T, Tian H, Ma Y, Xu H, Yao D, Loor JJ: MicroRNA-26a/b and their host genes synergistically regulate triacylglycerol synthesis by targeting the INSIG1 gene. RNA Biology 2016:1-11.

[100] Romao JM, Jin W, He M, McAllister T, Guan LL: Altered microRNA expression in bovine subcutaneous and visceral adipose tissues from cattle under different diet. PLoS One 2012, 7:e40605 (10 pages).

[101] Meale S, Romao J, He M, Chaves A, McAllister T, Guan L: Effect of diet on microRNA expression in ovine subcutaneous and visceral adipose tissues. Journal of Animal Science 2014, **92**(8):3328-3337.

[102] Romao J, Jin W, He M, McAllister T, Guan L: MicroRNAs in bovine adipogenesis: Genomic context, expression and function. BMC Genomics 2014, 15:137 (15 pages).

[103] Fatima A, Waters S, O'Boyle P, Seoighe C, Morris DG: Alterations in hepatic miRNA expression during negative energy balance in postpartum dairy cattle. BMC Genomics 2014, **15**(1):1.

[104] Wang D, Liang G, Wang B, Sun H, Liu J, Guan LL: Systematic microRNAome profiling reveals the roles of microRNAs in milk protein metabolism and quality: Insights on low-quality forage utilization. Scientific Reports 2016, 6: 21194 (16 pages).

[105] Mobuchon L, Marthey S, Le Guillou S, Laloë D, Le Provost F, Leroux C: Food deprivation affects the miRNome in the lactating goat mammary gland. PLoS One 2015, **10**(10):e0140111.

[106] Lawless N, Vegh P, O'Farrelly C, Lynn DJ: The Role of microRNAs in Bovine Infection and Immunity. Frontiers in Immunology 2014, 5:611 (7 pages).

[107] Lawless N, Foroushani AB, McCabe MS, O'Farrelly C, Lynn DJ: Next generation sequencing reveals the expression of a unique miRNA profile in response to a gram-positive bacterial infection. PLoS One 2013, **8**(3):e57543.

[108] Jin W, Ibeagha-Awemu EM, Liang G, Beaudoin F, Zhao X: Transcriptome microRNA profiling of bovine mammary epithelial cells challenged with Escherichia coli or Staphylococcus aureus bacteria reveals pathogen directed microRNA expression profiles. BMC Genomics 2014, 15: 181 (16 pages).

[109] Vegh P, Foroushani AB, Magee DA, McCabe MS, Browne JA, Nalpas NC, Conlon KM, Gordon SV, Bradley DG, MacHugh DE: Profiling microRNA expression in bovine alveolar macrophages using RNA-seq. Veterinary Immunology And Immunopathology 2013, **155**(4):238-244.

[110] Lawless N, Reinhardt TA, Bryan K, Baker M, Pesch B, Zimmerman D, Zuelke K, Sonstegard T, O'Farrelly C, Lippolis JD: MicroRNA regulation of bovine monocyte inflammatory and metabolic networks in an in vivo infection model. G3: Genes| Genomes| Genetics 2014, **4**(6):957-971.

[111] Li R, Zhang C-L, Liao X-X, Chen D, Wang W-Q, Zhu Y-H, Geng X-H, Ji D-J, Mao Y-J, Gong Y-C: Transcriptome microRNA profiling of bovine mammary glands infected with Staphylococcus aureus. International Journal of Molecular Sciences 2015, **16**(3):4997-5013.

[112] Liang G, Malmuthuge N, Griebel P: Model systems to analyze the role of miRNAs and commensal microflora in bovine mucosal immune system development. Molecular Immunology 2015, **66**(1):57-67.

[113] Salilew-Wondim D, Ibrahim S, Gebremedhn S, Tesfaye D, Heppelmann M, Bollwein H, Pfarrer C, Tholen E, Neuhoff C, Schellander K: Clinical and subclinical endometritis induced alterations in bovine endometrial transcriptome and miRNome profile. BMC Genomics 2016, **17**(1):1.

[114] Naeem A, Zhong K, Moisá S, Drackley J, Moyes K, Loor J: Bioinformatics analysis of microRNA and putative target genes in bovine mammary tissue infected with Streptococcus uberis. Journal of Dairy Science 2012, **95**(11):6397-6408.

[115] Hou Q, Huang J, Ju Z, Li Q, Li L, Wang C, Sun T, Wang L, Hou M, Hang S: Identification of splice variants, targeted microRNAs and functional single nucleotide polymorphisms of the BOLA-DQA2 gene in dairy cattle. DNA and Cell Biology 2012, **31**(5):739-744.

[116] Li L, Huang J, Zhang X, Ju Z, Qi C, Zhang Y, Li Q, Wang C, Miao W, Zhong J: One SNP in the 3′-UTR of HMGB1 gene affects the binding of target bta-miR-223 and is involved in mastitis in dairy cattle. Immunogenetics 2012, **64**(11):817-824.

[117] Zhang L, Hou D, Chen X, Li D, Zhu L, Zhang Y, Li J, Bian Z, Liang X, Cai X: Exogenous plant MIR168a specifically targets mammalian LDLRAP1: Evidence of cross-kingdom regulation by microRNA. Cell Research 2012, **22**(1):107-126.

[118] Baier SR, Nguyen C, Xie F, Wood JR, Zempleni J: MicroRNAs are absorbed in biologically meaningful amounts from nutritionally relevant doses of cow milk and affect gene expression in peripheral blood mononuclear cells, HEK-293 kidney cell cultures, and mouse livers. The Journal of Nutrition 2014, **144**(10):1495-1500.

[119] Liang H, Huang L, Cao J, Zen K, Chen X, Zhang CY: Regulation of mammalian gene expression by exogenous microRNAs. Wiley Interdisciplinary Reviews: RNA 2012, **3**(5):733-742.

[120] Denzler R, Stoffel M: Uptake and function studies of maternal milk-derived microRNAs. Journal of Biological Chemistry 2015, **290**(39):23680-23691.

[121] Van Rooij E, Bushell M, Zhang CY, Dashwood R, James W, Harris C, Baltimore D: The role of microRNA in nutritional control. Journal of Internal Medicine 2015, **278**(2):99-109.

[122] Fabris L, Calin GA: Circulating free xeno-microRNAs—The new kids on the block. Molecular Oncology 2016, **10**(3):503-508.

[123] Hirschi KD, Pruss GJ, Vance V: Dietary delivery: A new avenue for microRNA therapeutics? Trends in Biotechnology 2015, **33**(8):431-432.

[124] Wolf T, Baier SR, Zempleni J: The intestinal transport of bovine milk exosomes is mediated by endocytosis in human colon carcinoma Caco-2 cells and rat small intestinal IEC-6 cells. The Journal of Nutrition 2015, **145**(10):2201-2206.

[125] Izumi H, Tsuda M, Sato Y, Kosaka N, Ochiya T, Iwamoto H, Namba K, Takeda Y: Bovine milk exosomes contain microRNA and mRNA and are taken up by human macrophages. Journal of Dairy Science 2015, **98**(5):2920-2933.

[126] Micó V, Martín R, Lasunción MA, Ordovás JM, Daimiel L: Unsuccessful detection of plant microRNAs in beer, extra virgin olive oil and human plasma after an acute ingestion of extra virgin olive oil. Plant Foods for Human Nutrition 2016, **71**(1):102-108.

[127] Melnik BC, Kakulas F, Geddes DT, Hartmann PE, John SM, Carrera-Bastos P, Cordain L, Schmitz G: Milk miRNAs: Simple nutrients or systemic functional regulators? Nutrition & Metabolism 2016, **13**(1):1.

[128] Masood M, Everett CP, Chan SY, Snow JW: Negligible uptake and transfer of diet-derived pollen microRNAs in adult honey bees. RNA biology 2016, **13**(1):109-118.

[129] Auerbach A, Vyas G, Li A, Halushka M, Witwer K: Uptake of dietary milk miRNAs by adult humans: A validation study. F1000Research 2016, **5**:721.

[130] Huang W, Long N, Khatib H: Genome-wide identification and initial characterization of bovine long non-coding RNAs from EST data. Animal Genetics 2012, **43**(6):674-682.

[131] Weikard R, Hadlich F, Kuehn C: Identification of novel transcripts and noncoding RNAs in bovine skin by deep next generation sequencing. BMC Genomics 2013, 14:789 (15 pages).

[132] Billerey C, Boussaha M, Esquerré D, Rebours E, Djari A, Meersseman C, Klopp C, Gautheret D, Rocha D: Identification of large intergenic non-coding RNAs in bovine muscle using next-generation transcriptomic sequencing. BMC Genomics 2014, 15:499 (10 pages).

[133] Koufariotis LT, Chen Y-PP, Chamberlain A, Vander Jagt C, Hayes BJ: A catalogue of novel bovine long noncoding RNA across 18 tissues. PLoS One 2015, **10**(10):e0141225.

[134] Ibeagha-Awemu EM, Li R, Dudemaine P-L: The long non-coding RNA transcriptome of the bovine mammary gland and potential regulatory roles in fatty acid synthesis. Proceedings of the 6th International Symposium on Animal Functional Genomics

(6th ISFAG) 2015, Piacenza, 91. Available at: http://www.isafg2015.it/ISAFG2015_PROCEEDINGS.pdf.

[135] Zhan S, Dong Y, Zhao W, Guo J, Zhong T, Wang L, Li L, Zhang H: Genome-wide identification and characterization of long non-coding RNAs in developmental skeletal muscle of fetal goat. BMC Genomics 2016, **17**(1):666.

[136] Ren H, Wang G, Chen L, Jiang J, Liu L, Li N, Zhao J, Sun X, Zhou P: Genome-wide analysis of long non-coding RNAs at early stage of skin pigmentation in goats (Capra hircus). BMC Genomics 2016, **17**(1):67.

[137] Chao T, Wang G, Wang J, Liu Z, Ji Z, Hou L, Zhang C: Identification and classification of new transcripts in Dorper and small-tailed Han sheep skeletal muscle transcriptomes. PLoS One 2016, **11**(7):e0159638.

[138] Wapinski O, Chang HY: Long noncoding RNAs and human disease. Trends in Cell Biology 2011, **21**(6):354-361.

[139] Cheetham S, Gruhl F, Mattick J, Dinger M: Long noncoding RNAs and the genetics of cancer. British Journal of Cancer 2013, **108**(12):2419-2425.

[140] Esteller M: Non-coding RNAs in human disease. Nature Reviews Genetics 2011, **12**(12): 861-874.

[141] Atianand MK, Fitzgerald KA: Long non-coding RNAs and control of gene expression in the immune system. Trends in Molecular Medicine 2014, **20**(11):623-631.

[142] Devaux Y, Zangrando J, Schroen B, Creemers EE, Pedrazzini T, Chang C-P, Dorn II GW, Thum T, Heymans S: Long noncoding RNAs in cardiac development and ageing. Nature Reviews Cardiology 2015, **12**(7):415-425.

[143] Kim J, Kim KM, Noh JH, Yoon J-H, Abdelmohsen K, Gorospe M: Long noncoding RNAs in diseases of aging. Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms 2016, **1859**(1):209-221.

[144] Yu L, Batara J, Lu B: Application of genome editing technology to microRNA research in mammalians; 2016. Modern Tools for Genetic Engineering, InTech, Rijeka, Croatia, DOI: 10.5772/64330.

[145] Laible G, Wei J, Wagner S: Improving livestock for agriculture–technological progress from random transgenesis to precision genome editing heralds a new era. Biotechnology Journal 2015, **10**(1):109-120.

[146] West J, Gill WW: Genome editing in large animals. Journal of Equine Veterinary Science 2016, **41**:1-6.

[147] Tizard M, Hallerman E, Fahrenkrug S, Newell-McGloughlin M, Gibson J, de Loos F, Wagner S, Laible G, Han JY, D'Occio M: Strategies to enable the adoption of animal biotechnology to sustainably improve global food safety and security. Transgenic Research 2016:1-21.

[148] Proudfoot C, Carlson DF, Huddart R, Long CR, Pryor JH, King TJ, Lillico SG, Mileham AJ, McLaren DG, Whitelaw CBA: Genome edited sheep and cattle. Transgenic Research 2015, **24**(1):147-153.

[149] Carlson DF, Walton MW, Fahrenkrug SC, Hackett PB: Precision editing of large animal genomes. Advances in Genetics 2012, **80**:37.

[150] Butler JR, Ladowski JM, Martens GR, Tector M, Tector AJ: Recent advances in genome editing and creation of genetically modified pigs. International Journal of Surgery 2015, **23**:217-222.

[151] Kim Y-G, Cha J, Chandrasegaran S: Hybrid restriction enzymes: Zinc finger fusions to Fok I cleavage domain. Proceedings of the National Academy of Sciences 1996, **93**(3):1156-1160.

[152] Boch J, Scholze H, Schornack S, Landgraf A, Hahn S, Kay S, Lahaye T, Nickstadt A, Bonas U: Breaking the code of DNA binding specificity of TAL-type III effectors. Science 2009, **326**(5959):1509-1512.

[153] Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E: A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. Science 2012, **337**(6096):816-821.

[154] Lillico SG, Proudfoot C, Carlson DF, Stverakova D, Neil C, Blain C, King TJ, Ritchie WA, Tan W, Mileham AJ: Live pigs produced from genome edited zygotes. Scientific Reports 2013, **3**:2847.

[155] Whitworth KM, Lee K, Benne JA, Beaton BP, Spate LD, Murphy SL, Samuel MS, Mao J, O'Gorman C, Walters EM: Use of the CRISPR/Cas9 system to produce genetically engineered pigs from in vitro-derived oocytes and embryos. Biology of Reproduction 2014, **91**(3):78.

[156] Ge H, Cui C, Liu J, Luo Y, Quan F, Jin Y, Zhang Y: The growth and reproduction performance of TALEN-mediated β-lactoglobulin-knockout bucks. Transgenic Research 2016:1-9.

[157] Wu H, Wang Y, Zhang Y, Yang M, Lv J, Liu J, Zhang Y: TALE nickase-mediated SP110 knockin endows cattle with increased resistance to tuberculosis. Proceedings of the National Academy of Sciences 2015, **112**(13):E1530-E1539.

[158] Zhang C, Wang L, Ren G, Li Z, Ren C, Zhang T, Xu K, Zhang Z: Targeted disruption of the sheep MSTN gene by engineered zinc-finger nucleases. Molecular Biology Reports 2014, **41**(1):209-215.

[159] Cui C, Song Y, Liu J, Ge H, Li Q, Huang H, Hu L, Zhu H, Jin Y, Zhang Y: Gene targeting by TALEN-induced homologous recombination in goats directs production of β-lactoglobulin-free, high-human lactoferrin milk. Scientific Reports 2015, **5**:10482.

[160] Yu S, Luo J, Song Z, Ding F, Dai Y, Li N: Highly efficient modification of beta-lactoglobulin (BLG) gene via zinc-finger nucleases in cattle. Cell Research 2011, **21**(11):1638.

[161] Liu X, Wang Y, Guo W, Chang B, Liu J, Guo Z, Quan F, Zhang Y: Zinc-finger nickas-emediated insertion of the lysostaphin gene into the beta-casein locus in cloned cows. Nature Communications 2013, 4:2565 (11 pages).

[162] Liu X, Wang Y, Tian Y, Yu Y, Gao M, Hu G, Su F, Pan S, Luo Y, Guo Z: Generation of mastitis resistance in cows by targeting human lysozyme gene to β-casein locus using zinc-finger nucleases. Proceedings of the Royal Society B 2014, 281:20133368 (10 pages).

[163] Jenko J, Gorjanc G, Cleveland MA, Varshney RK, Whitelaw CBA, Woolliams JA, Hickey JM: Potential of promotion of alleles by genome editing to improve quantitative traits in livestock breeding programs. Genetics Selection Evolution 2015, **47**(1):55.

[164] Chen B, Chen X, Wu X, Wang X, Wang Y, Lin T-Y, Kurata J, Wu J, Vonderfecht S, Sun G et al: Disruption of microRNA-21 by TALEN leads to diminished cell transformation and increased expression of cell–environment interaction genes. Cancer Letters 2015, **356**(2, Part B):506-516.

[165] Jabed A, Wagner S, McCracken J, Wells DN, Laible G: Targeted microRNA expression in dairy cattle directs production of β-lactoglobulin-free, high-casein milk. Proceedings of the National Academy of Sciences 2012, **109**(42):16811-16816.

IntechOpen