J. Dairy Sci. 95:6529–6535
http://dx.doi.org/10.3168/jds.2012-5748

MicroRNA expression patterns in the bovine mammary gland are affected by stage of lactation[1]

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ABSTRACT

The objective of this work was to determine the expression pattern of microRNA (miR) associated with cellular proliferation, lipid metabolism, and innate immunity in dairy cow mammary gland tissue at different stages of lactation. The expression of miR-10a, miR-15b, miR-16, miR-21, miR-31, miR-33b, miR-145, miR-146b, miR-155, miR-181a, miR-205, miR-221, and miR-223 was studied by real-time reverse-transcription PCR in tissue (n = 7/stage) harvested via repeated biopsies during the dry period (−30 d prepartum), the fresh period (7 d postpartum), and early lactation (30 d postpartum). Except for miR-31, all miR studied increased in expression between the dry and fresh periods. Among those upregulated, the expression of miR-221 increased further at early lactation, suggesting a role in the control of endothelial cell proliferation or angiogenesis, whereas the expression of miR-223 decreased at early lactation but to a level that was greater than in the dry period, suggesting it could play a role in the mammary response to pathogens soon after parturition. The expression of miR-31, a hormonally regulated miR that inhibits cyclin gene expression, was greater at early lactation compared with the dry period. From a metabolic standpoint, the consistent upregulation of miR-33b during early lactation compared with the dry period suggests that this miR may exert some control over lipogenesis in mammary tissue. Overall, results indicate that expression of miR associated with transcriptional regulation of genes across diverse biological functions is altered by stage of lactation. The specific roles of these miR during lactation will require further research.

Key words: dairy cow, mammary gland, lactation, microribonucleic acid

INTRODUCTION

MicroRNA (**miR**) are small noncoding RNA that regulate gene expression posttranscriptionally and play a key role in development and specific biological processes, such as cell proliferation, differentiation, and apoptosis, in several species (Filipowicz et al., 2008). Work in the mouse recently revealed that tissues have a specific miRNome pattern of expression, that is, a full complement of miR (Liu et al., 2004). The human breast-specific signature is characterized by the expression of 23 miR (miR-let-7a-1, miR-let-7b, miR-023a, miR-023b, miR-024-2, miR-026a, miR-026b, miR-030b, miR-030c, miR-030d, miR-092-1, miR-092-2, miR-100-1/2, miR-103-1, miR-107, miR-146, miR-191, miR-197, miR-205, miR-206, miR-213, miR-214, and miR-221) out of 161 studied (Liu et al., 2004), and the mouse mammary-specific signature is characterized by the expression of 9 miR (miR-let-7a, miR-let-7b, miR-let-7c, miR- 26a, miR-26b, miR-24-2, miR-145, miR-30b, and miR-30d) out of 22 detected (Silveri et al., 2006), suggesting that miR could play a role in mammary gland physiology.

The mammary gland is unique in its capability to undergo cycles of cell proliferation, differentiation, and apoptosis during adult life. The complex regulation of mammary development has been extensively studied over the years at the genetic, physiological, and morphological levels (Anderson et al., 2007). Because of the relatively recent recognition of miR as key regulators of cellular function, only a few reports have focused on the role of miR in normal mammary development. To our knowledge, the longitudinal changes in miR expression profiles during different stages of lactation have been evaluated only in the mouse (Silveri et al., 2006). Therefore, knowledge of the expression patterns of miR in the bovine mammary gland during the transition from pregnancy into lactation might provide insight into their roles in such functions as regulation of me-

Received May 18, 2012.
Accepted July 26, 2012.
[1]Financial support for the study was provided by the National Key Basic Research Program of China (project number 2011CB100805) and by the Cooperative State Research, Education and Extension Service (USDA, Washington, DC; project number ILLU-538-307).
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tabolism, angiogenesis, differentiation and apoptosis, and the immune response.

The general hypothesis of the present study was that the expression of selected miR associated with cellular proliferation, metabolism, and innate immune response differs between the dry period and lactation. Furthermore, such patterns of expression may be associated with the mRNA expression of metabolic (Bionaz and Loor, 2008, 2011), cell proliferation and apoptosis (Annen et al., 2008), angiogenesis (Mattmiller et al., 2011), apoptosis, and immune or stress-related genes (Aitken et al., 2009). Therefore, the main objective of this work was to determine the expression pattern of several important miR in the Holstein cow mammary gland at different stages of the lactation cycle (dry period, fresh period after calving, and early lactation), and to provide the basis for selection of candidate miR for more detailed functional characterization in the future.

MATERIALS AND METHODS

All procedures involving animals received approval from the Institutional Animal Care and Use Committee at the University of Illinois, Urbana (protocol 06145). The right rear quarter of 7 cows was biopsied at −30 and 30 d, and the left rear quarter was biopsied at 7 d. The midsections of the rear quarters were selected for the initial biopsy. Subsequently, a different section located approximately 5 cm from the original incision site of the right quarter was selected for biopsy. Biopsies were conducted at approximately 0700 h (after the morning milking). Briefly, after the skin incision was made, blunt dissection of the mammary capsule was performed to ensure the tissue obtained during the biopsy was mammary parenchyma; that is, biopsies were performed once the view of the mammary capsule was clear such that the tip of the biopsy tool could go through the dissected connective tissue. Immediately after removal of the biopsy instrument from the capsule, pressure was applied to the wound until visual signs of bleeding were absent. The skin incision was closed with 4 or 5 Michel clips (11 mm; Henry Schein, Melville, NY). The incision site was sprayed with topical antiseptic (10% Povidone Iodine Ointment; Taro Pharmaceuticals, Hawthorne, NY).

Samples were frozen in liquid nitrogen within 30 s after tissue dissection. Frozen samples were stored at −80°C. Later, mammary gland tissue (100 mg) was homogenized in lysis/binding buffer with 1/10 vol of homogenate additive (mir-Vana miRNA Isolation Kit, AM1561; Applied Biosystems, Foster City, CA) using a Tissue-Tearor (BioSpec Products, Bartlesville, OK) homogenizer. After prehomogenizing samples at 10,000 rpm at 4°C for 10 min to remove fat and cell debris, miR were extracted from the supernatant according to the manufacturer's protocol (mir-Vana miRNA Isolation Kit, AM1561; Applied Biosystems). Ribonucleic acid (10 μg) was polyadenylated using Poly(A) polymerase according to the manufacturer's instructions (NCode miRNA, First Strand cDNA Module, no. 45-6612; Invitrogen, Foster City, CA). Complementary DNA was made as follows: 10 μg of polyadenylated RNA was reverse-transcribed using Superscript II reverse transcriptase (Invitrogen) with 2.5 μg of random hexamers and 500 ng of oligo(dT) adapter primer (Invitrogen), according to the manufacturer's instructions.

The reactions were performed in triplicate as follows: 5 μL of cDNA were mixed with 5 pmol of both the forward (F) and reverse (R) primers in a final volume of 12.5 μL and mixed with 12.5 μL of 2× Power SYBR Green PCR Master Mix (No. 4367659; Applied Biosystems). All reactions were run using 2 amplification protocols: 20 s at 94°C, 30 s at 59°C, and 20 s at 72°C for 40 cycles. The same conditions were performed on an equal amount of RNase-free water as a negative control. Primer sequences were as follows: miR-10a-F: ACCCTGTAGATCCGAATTTGTG (NR_031364.1); miR-145-F: CCAGTTTTCCCAGGAATCCCT (NR_030906.1); miR-146b-F: TGAGAACTGAATTCCATAGGC (NR_031033.1); miR-155-F: TTAATGCTAATCGTGATAGGGG (NR_031030.1); miR-15b-F: TAGCAGCACATCATGGTTTACA (NR_031363.1); miR-16-F: TAGCAGCACGTAAATATTGGC (NR_030891.1); miR-181a-F: ATTCAACGCTGTCGGTGAGTT (NR_031081.1); miR-205-F: TCCTTCATTCCACCGGAGTCT (NR_030909.1); miR-21-F: GCTTATCAGACTGATGTTGACT (NR_030880.1); miR-221-F: AGCTACATTGTCTGCTGGGTTT (NR_030881.1); miR223-F: TGTCAGTTTGTCAAATACCCCA (NR_031144.1); miR-31-F: AGGCAAGATGCTGGCATAGCT (DQ274883.1); miR-33b-F: GTGCATTGCTGTTGCATTG (NR_031207.1); U6 (internal control miR)-F: CGCTTCGGCAGCACATATAC; and U6-R: TCACGAATTTGCGTGTCAT. We also analyzed expression of miR-146a, miR-17, and miR-33a; they were undetectable under the conditions used and will not be discussed further. Real-time quantitative PCR with SYBR Green I (Power SYBR Green, PCR Master Mix, No. 4367659; Applied Biosystems) was performed in an ABI Prism 7900 HT SDS instrument (Applied Biosystems), and the data were calculated with 7900 HT Sequence Detection Systems software (version 2.2.1; Applied Biosystems). The geometric mean of 5S (catalog no. AM30302; Applied Biosystems) and U6 was used to normalize the expression of target miR (Gandellini et al., 2009; Xu et al., 2010).