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Mechanistic Effects of microRNA in Pathogenesis of Bovine Mastitis

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ABSTRACT

Mastitis is a multietiological complex disease, characterized by the inflammation of parenchyma of mammary glands there by changing the physical, chemical and bacteriological properties of milk. Bovine mastitis also causes pathological changes in glandular tissues. In the last few years a no of RNA molecules have been discovered and as a result of this the RNA family has been rapidly growing. Of all these RNA molecules that have been found specific attention has been to given to a special class of RNA molecules known as MicroRNAs (mi-RNAs /miRs) .This particular class of RNA molecule has been given more attention because of the fact that they act as powerful regulators of gene expression. Also this class of RNA molecule shows its fundamental impact on the pathogenesis of different pathological events. MicroRNAs (mi-RNAs) are a group of endogenous non-coding small RNA molecules, usually 20-25 nucleotides in length. MicroRNAs (mi-RNAs) play an important role in the regulation of gene expression for important biological processes like cellular proliferation and differentiation, tissue development and immune response. MicroRNAs (mi-RNAs) regulate gene expression post-transcriptionally and are found to be important regulators of epithelial immune responses. Therefore it has became more important to find whether there is any role of mi-RNAs in bovine mastitis or not and also its association to mastitis. Further studies in future needs to be carried out suggest the potential utility of mi-RNAs to serve as biomarkers for the diagnosis of mastitis in dairy cows. The application of mi-RNAs as a potential biomarker has shed light as a tool for detection and verification of a disease in animals.

Keywords : Mastitis, MicroRNAs (mi-RNAs), gene regulation and biomarkers.

I. INTRODUCTION

Bovine mastitis is an inflammatory disease of the bovine mammary gland and is regarded as an important health issue in dairy cattle. Bovine mastitis causes great economic loses to the global dairy industry. Mastitis is a multi-factorial disease and the main etiological agents are the bacteria. Mastitis usually develops when these bacteria enter the udder via teat canal. The epithelial cells line the mammary gland and when the pathogens like bacteria enter the body these cells stimulate a local inflammatory response which ultimately facilitates their transport across the epithelial barrier where they are detected by resident immune cells, such as monocytes. These cells on their surfaces have pathogen recognition molecules like Toll-like receptors (TLRs), enabling them to function in a sentinel capacity. Invasive S. uberis releases TLR2 and TLR4 mobilizing local and systemic inflammatory mediators (Moyes *et al*. 2009).The release of certain inflammatory factors like chemokines, interleukins (ILs), and tumour necrosis factor (TNF)-a causes changes in vascular permeability, cell differentiation, and apoptosis. The changes in the systemic innate immunity trigger the production of acute phase protein (APP) production, which is distributed systemically to suppress the spread of bacteria locally (Mitterhuemer *et al*. 2010).Both in vivo and in vitro a number of approaches have been utilized to examine the immune response in mammary tissues. However not enough of data is available to measure transcriptional

activity in milk and blood monocytes from infected animals (Prgomet *et al.* 2005) and whether micro-RNAs (mi-RNAs) do play a role in the regulation of these responses is still insignificant.

The mi-RNAs are usually small, non-coding RNAs that play a significant role in regulating innate and adaptive immunity as they act as posttranscriptional regulators of gene expression (O'Connell *et al*. 2010). In several types of cells, micro-RNAs regulate immune function as senescence of neutrophils is regulated by discrete mi-RNA repertoire(Ward *et al.* 2011). In the tissues of numerous bovine, the expression of mi-RNA is abundant suggesting the limited role of mi-RNA in bovine immunity (Xu *et al*. 2009)

Micro RNAs (mi-RNAs) have long been the popular issues for the studying of disease versus normal health statuses in human medicine. In that way, mi-RNAs can be utilized for the detection of prostate cancer (Mitchell *et al.,* 2008), breast cancer (Zhao *et al.,* 2010) and heart disease as biomarkers in human (Goren et al., 2012). Presently mi-RNAs in cow have been confirmed, characterized, and fully elucidated for their presence (Fatima and Morris, 2013). The presence of specific mi-RNAs in body fluids such as blood and milk may be utilized as a biomarker for diagnosis of mastitis particularly those mi-RNAs that are associated with mastitis status. During the adult life, the mammary gland has a unique capability to undergo cycles of cell proliferation, differentiation, and apoptosis. Over the years studies at genetic, physiological, and morphological levels have been carried out how they regulate the development of mammary gland(Anderson *et al*., 2007).Due to these recent studies which focused on that mi-RNAs could act as key regulators of cellular function, only a few studies have suggested that mi-RNAs have a role in the normal mammary development. The transition period from pregnancy into lactation may provide knowledge about the pattern of mi-RNA expression in bovine mammary gland which ultimately could determine their roles in such functions as regulation of metabolism, angiogenesis, differentiation and apoptosis and the immune response.

MicroRNA biogenesis

Micro RNA (mi-RNAs)are a class of endogenous non-coding RNAs (~22 nucleotides) which usually bind to the 3'UTR of target mRNAs to repress translation and/or accelerate the decay of up to 30 % of all expressed transcripts. In different species mi-RNAs are highly conserved and are thought to regulate at least 50% of the genome. Mi-RNAs act as posttranscriptional regulators of gene expression thereby regulating innate as well as adaptive immunity (O'Connell *et al*. 2010). The functions of mi-RNA involves post-transcriptional control of gene expression which occurs either by protein translational repression or by promoting mRNA degradation. Mi-RNAs are found in a variety of organisms ranging from plants to animals. It has been reported that in plants, invertebrates, vertebrates, as well as in mammals, mi-RNAs play an important role in many developmental and cellular processes (Bartel, 2004).

The mi-RNAs are produced by various types of cells and these are finally secreted into extracellular spaces and fluids. Within the genome mi-RNA genes are located in separate segments or situated as part of transcriptional gene, especially within intron of genes (Mirtrons). biogenesis of mi-RNAs occurs in nuclei from the hairpin configuration of the primary transcript (pri-miRNA) which is larger than functional mature mi-RNA (O'Connell *et al.,* 2012). The latter process involves cleavage of a large hairpin structure to small hairpin (pre-mi-RNA) by the activities of Drosha-DGCR8 (DiGeorge critical region 8) exonuclease enzymes . After trimming, short RNA structures (pre-mi-RNA) are then exported out to cytoplasm assists by Exportin-5 (Krol *et al.,* 2010). Further process has been made inside the cytosol to create a functional mature mi-RNA by Dicer and TRBP (Tar RNA binding protein) proteins. The mi-RNA duplexes are loaded into Argonaute protein (Ago2) in effector complexes, also known as, RNA-induced silencing complex (RISC) (Winter *et al*., 2009). One of the thermodynamically less stable at 5'-end of the two strands in the duplex will become the mature mi-RNA. Then, mature mi-RNA is preferentially retained in RISC and form miRISCs complex resulting in their inhibition (Pasquinelli, 2012).

MicroRNAs in cow genome

Mi-RNA are found to be present in the genome of cows. It has been studied and reported that the genome of cows contain 793 mi-RNAs which in turn are encoded by 30 chromosomes(Fatima and Morris, 2013).According to the studies carried out by Flicek *et al*., 2014 about 3,825 non-coding RNAs in cow whole genome can be located.

The nucleotide sequences of cattle bovine-mir-155 is evolutionarily conserved across human and mammalian species. The presence of this identical nucleotide sequence makes them recognize a similar mi-RNA target recognition site. Micro RNAs found in different species are homologous and appear to perform the same function. As hsa-miR-155 is found in humans and bta-miR-155 in cattle are homologous and play a vital role in inflammatory processes. In humans hsa-miR-146a-5p which helps in regulating the expression of retinoic acid-inducible gene 1 (RIG-I) is found to be orthologous in bovine (Hou *et al.,* 2009).Also other mi-RNAs , such as, bta-miR-2284 family are discovered only in cows.

MiRNA as Biological markers

The use of specific biomarkers can play a crucial role in the evaluation of normal or disease status in cows. The utility of using mi-RNAs in human medicine as indicators have already been proven because of its property to detect the biologically relevant to specimens for the non-invasive procedure (Chen *et al*., 2012).Making use of these properties mi-RNA can also prove a very useful biomarker in mastitis. When mi-RNA accumulates in extracellular fluids, such as plasma, serum, milk, urine, seminal fluids, it has a very stable property and can be easily detected (Weber *et al*., 2010).Mi-RNAs can be utilized as a potential biomarker for detection and diagnosis of bovine mastitis. A number of mi-RNA have been found in milk and these include miR-10a, miR-15b, miR-21, miR-33b, miR-145, miR-146b, miR-155, miR-181a, miR-205, miR-221, and miR-223 are present in milk (Wang *et al*., 2012). The mi-RNAs like miR-146a and miR-223 which are found to be associated with milk have some potentials in using as a biomarker of ongoing bacteria-causing bovine mastitis. Within the exosome, several mi-RNAs in milk can be protected securely (Melnik *et al*., 2014). In some instances mi-RNA aids in the defense mechanism of hosts by being a part of the immune system(Sun *et al*., 2015). Raw milk, colostrum, or milk-related products such as milk powder contains mi-RNAs. These mi-RNAs are found to be highly stable in such harsh environment and condition (Howard *et al.,* 2015). In milk a large number of specific mi-RNAs are found which are sometimes called as"milk-associated mi-RNAs" and these include miR-26a, miR-26b, miR-200c, miR-21, miR-30d, miR-99a and miR-148a (Chen *et al.,* 2010). Previous studies have reported that a collection of mi-RNAs is linked to bacteria-causing mastitis in cows (Lawless *et al.,* 2014). Hence, the detection of mi-RNAs in milks is to be used as a candidate diagnostic tool for an early indication of bovine mastitis, if applicable. This will make it possible as a time saver, high accuracy technique, cheap, reliable and easily available method for the of detection mastitis.

II. FUTURE RESEARCH

It is clear that in human and mouse mi-RNAs play a key role in the regulation of immune responses. It is very important from the scientific point of view that how do mi-RNAs regulate gene expression in bovines. The process of using mi-RNAs as biomarkers could be of great importance in the diagnosis and detection of specific diseases. According to various studies carried out in vitro, there is an association between mi-RNA expression and different mastitis causing pathogens. Due to a limited number of studies carried out in vivo the utility of using mi- RNAs as biomarkers are limited. In this regard, studies need to be carried out on a large scale so that the potentiality of using mi-RNAs as biomarkers are significant and of utmost importance. In particular infections like tuberculosis, in vivo studies, could be very useful to identify sensitive and specific biomarkers. Further, the studies which have been carried out on the role of mi-RNAs in regulating immunity of bovines has mainly focused on bacterial infections while as little knowledge is available regarding the role of mi-RNAs in bovine viral infections. It should be kept in view that mi-RNA besides being used as biomarkers can also be explored as therapeutic targets or agents. Several mi-RNAs in humans like miR-122 and miR-208 are being used as novel therapeutics in cancer, viral infections, and cardiovascular disease. This mi- RNAs have orthologs in cattle which clearly suggests that there is a potential of being used for therapeutic purposes in order to combat diseases and regulate metabolism.

III. CONCLUSION

In conclusion, it is clear that mi-RNAs significantly play a key role in the regulation of immunity in bovine. Further studies in this regard need to be carried out which could truly prove their potential of being a novel biomarker and therapeutic agents in bovine. Also more research and studies on how they regulate gene

expression in bovine needs to be explored for the welfare of animal health.

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