Signal Transduction Mechanism of Decreased Milk Protein Content in Dairy Cows with Subclinical Mastitis: Postprint

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Date: 2017-10-11T00:00:00+00:00

Abstract

Milk protein is one of the primary milk constituents that determines the nutritional quality of milk, with high nutritional value containing almost all essential amino acids required by the human body. The content and composition of milk protein are influenced by numerous factors. Apart from breed, parity, environment, lactation stage of dairy cows, and dietary composition and nutritional level, disease also represents an important factor affecting milk protein content and composition. Among these, subclinical mastitis in dairy cows can lead to reduced milk protein content. This article primarily investigates the synthesis mechanism of milk protein and the signal transduction mechanisms underlying milk protein content reduction during subclinical mastitis, aiming to provide insights and references for research on methods to enhance milk protein content under conditions of subclinical mastitis in dairy cows.

Full Text

Signal Transduction Mechanism of Milk Protein Content Depression Induced by Subclinical Mastitis in Dairy Cows

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Abstract

Milk protein is one of the primary components determining the nutritional quality of milk, possessing high nutritional value and containing nearly all essential

amino acids required by humans. Both the content and composition of milk protein are influenced by multiple factors, including breed, parity, environment, lactation stage, dietary composition, and nutrient level. Additionally, disease represents an important factor affecting milk protein content and composition, with subclinical mastitis in dairy cows causing a reduction in milk protein content. This paper primarily explores the mechanisms of milk protein synthesis and the signal transduction pathways underlying reduced milk protein content during subclinical mastitis, aiming to provide insights and references for developing methods to improve milk protein content under subclinical mastitis conditions.

Keywords: dairy cow; subclinical mastitis; milk protein; signal transduction mechanism

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Milk protein constitutes a crucial material foundation for the nutritional quality of milk, containing nearly all essential amino acids and serving as an important source of dietary protein for humans. Milk protein content not only relates to milk quality, safety, and consumer health but also represents a key indicator of core competitiveness in the dairy industry. In recent years, with the development of the dairy industry and increasing emphasis on healthy consumption, demand for high-quality milk protein has continuously grown. Consequently, many countries worldwide now prioritize milk protein value in their milk pricing systems. China' s new pasteurized milk standard (GB 19645-2010) also lists milk protein ($ $2.9%) as an important quality indicator. Thus, increasing milk protein content and yield represents an inevitable trend in the dairy industry' s future development. However, after years of rapid quantitative expansion, China' s dairy industry faces the severe challenge of poor milk nutritional quality, particularly low milk protein content. The "melamine incident" triggered by this issue severely damaged consumer confidence in domestic dairy products and the healthy development of the dairy industry. Therefore, increasing milk protein content in milk is an urgent requirement for the healthy development of China' s dairy industry and holds significant importance for improving milk nutritional quality and enhancing the core competitiveness of the national dairy sector.

Milk protein content and composition are affected by numerous factors. Beyond breed, parity, environment, lactation stage, and dietary composition and nutrient level, disease also constitutes an important factor influencing milk protein content and composition. Among these, bovine mastitis significantly impacts milk protein content and composition. Mastitis is a common disease in dairy cows characterized by inflammatory responses in mammary tissue that reduce milk yield and quality, increase somatic cell count, decrease milk protein content, and cause substantial economic losses to modern dairy farming and milk processing industries. Subclinical mastitis, in particular, which has high incidence rates without obvious clinical symptoms, severely constrains improvements in milk protein content and yield. However, the molecular mechanisms by which

mammary gland infection reduces milk protein content remain incompletely understood. Therefore, this paper provides a preliminary exploration of the signal transduction mechanisms linking inflammatory responses during subclinical mastitis to reduced milk protein yield.

1. De novo Synthesis of Milk Protein

Milk protein is an important nutritional component in milk. In healthy dairy cows, milk protein content ranges from 3.0% to 3.7%. Milk proteins are primarily classified into two major categories: caseins (αS-casein, β-casein, γ-casein, and -casein) and whey proteins (WP), with small amounts of milk fat globule membrane protein (MFGMP) also present. β-casein serves as a key indicator for characterizing milk protein content. Over 90% of milk proteins, including caseins, α-lactalbumin, and β-lactoglobulin, are synthesized de novo in mammary tissue by mammary epithelial cells using amino acids absorbed from blood as substrates. The synthesis process involves several steps: mammary tissue absorbs amino acids from blood; various milk protein DNAs are transcribed into mRNA; tRNA carries free amino acids absorbed from blood by mammary tissue to ribosomes on the rough endoplasmic reticulum, where polypeptide chains are synthesized using ribonucleotide sequences on mRNA as templates; the nascent polypeptides are then guided by signal peptides into the endoplasmic reticulum lumen and undergo a series of chemical modifications including phosphorylation and glycosylation in the endoplasmic reticulum and Golgi apparatus to form mature proteins; finally, secretory vesicles transport these proteins to the apical membrane of mammary epithelial cells and release them into the mammary alveolar lumen via exocytosis. Any factor affecting any of these steps can influence milk protein content. For example, low dietary crude protein content reduces amino acid availability for mammary tissue, leading to significantly decreased milk protein yield.

2. Factors Regulating Milk Protein Gene Transcription and Translation

Milk protein gene transcription and translation play crucial roles in milk protein synthesis. In recent years, as research on functional regulation of mammary epithelial cells has gradually deepened, lactation cell signal transduction mechanisms have attracted widespread attention. Studies have identified two primary signaling pathways regulating milk protein synthesis: (1) the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway, which regulates milk protein gene transcription; and (2) the mammalian target of rapamycin (mTOR) pathway, which regulates milk protein gene translation.

2.1. Transcriptional Regulation of Milk Protein Genes

Milk protein gene transcription is a critical step in de novo milk protein synthesis, with the JAK-STAT signaling pathway playing an important regulatory

role at the transcriptional level. JAKs are a class of non-transmembrane tyrosine kinases comprising four family members: JAK1, JAK2, JAK3, and Tyk2. Activated JAKs can catalyze the phosphorylation of specific tyrosine residues on their associated receptors. STATs play important roles in signal transduction and transcriptional activation, with seven family members identified to date: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6. Bionaz et al. found that STAT5a and STAT5b are predominantly expressed in bovine mammary tissue. Various hormones and cytokines related to milk protein synthesis can regulate milk protein synthesis through the JAK-STAT signaling pathway. Recent research has focused primarily on the JAK2-STAT5 pathway. STAT5 serves as a marker transcription factor reflecting milk protein gene transcription levels, with JAK2 acting as its upstream signaling molecule. During lactation, hormones such as prolactin (PRL), growth hormone (GH), insulin (INS), and leptin, along with related cytokines, bind to their respective receptors on mammary epithelial cells, activating JAK2 and phosphorylating specific tyrosine residues on receptors to create binding sites for STAT5 and other intracellular signaling molecules. Activated JAK2 then phosphorylates recruited STAT5, which dissociates from the receptor, forms dimers via its Src homology 2 (SH2) domain, translocates to the nucleus, and binds to promoters of milk protein genes such as β-casein and -casein, initiating transcription and thereby participating in milk protein synthesis regulation.

2.2. Translational Regulation of Milk Protein Genes

Milk protein translation in mammary tissue is primarily regulated by the mTOR signaling pathway. mTOR is a structurally and functionally conserved atypical serine/threonine protein kinase belonging to the phosphatidylinositol kinase-related kinase (PIKK) family. The mTOR signaling protein exists in two complexes: rapamycin-sensitive mTOR complex 1 (mTORC1) and rapamycin-insensitive mTOR complex 2 (mTORC2). The mTOR pathway includes two upstream regulatory pathways—the phosphatidylinositol 3-kinase (PI3K)-protein kinase B (PKB or AKT)-mTOR pathway and the liver kinase B1 (LKB1)-AMP-activated protein kinase (AMPK)-mTOR pathway—and two downstream regulatory pathways: the eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1) pathway and the ribosomal protein S6 kinase (S6K) pathway. Amino acids such as leucine, isoleucine, and arginine, along with lactation-related hormones including growth hormone, insulin, and insulin-like growth factor I (IGF-I), can regulate milk protein synthesis by modulating mTOR phosphorylation levels and the activity of downstream signaling molecules 70 kDa ribosomal protein S6 kinase (p70S6K) and 4EBP1, thereby controlling translation of specific mRNA subgroups.

The PI3K-AKT-mTOR pathway plays the primary regulatory role in milk protein translation. PI3K is an intracellular phosphatidylinositol kinase with both serine/threonine kinase activity and phosphatidylinositol kinase activity. AKT is a serine/threonine protein kinase and an important downstream signaling

molecule of PI3K. After lactation-related hormones bind to their respective membrane receptors such as G protein-coupled receptors and protein tyrosine kinase receptors, PI3K is activated at the cell membrane, catalyzing the conversion of phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3). As a second messenger, PIP3 activates downstream AKT, which upon activation transmits signals to mTOR, causing its phosphorylation. Activated mTOR then phosphorylates downstream target proteins 4EBP1 and ribosomal protein S6 kinase 1 (S6K1), which are key regulators of protein translation. Unphosphorylated 4EBP1 binds to eukaryotic translation initiation factor 4E (eIF4E), inhibiting formation of the translation initiation complex and thereby suppressing protein translation. Unphosphorylated S6K1 remains inactive when bound to eukaryotic translation initiation factor 3 (eIF3). Studies have shown that upon signal-induced mTOR phosphorylation, the mTOR complex phosphorylates 4EBP1 at multiple sites, causing phosphorylated 4EBP1 to dissociate from eIF4E and release it. This increases available eIF4E, which then combines with other translation initiation factors to form the translation initiation complex, promoting mRNA translation initiation and protein synthesis. Additionally, phosphorylated mTOR complex can bind to eIF3, thereby activating and releasing S6K1 from eIF3. Activated S6K1 then phosphorylates downstream substrates such as ribosomal protein S6 (RPS6), enhancing the cell' s protein synthesis capacity and increasing protein yield.

3. Changes in Inflammatory Signaling Pathways During Subclinical Mastitis

Staphylococcus aureus-induced chronic infection is a major factor triggering subclinical mastitis in dairy cows. During S. aureus infection, lipoteichoic acid (LTA) on its surface acts as a ligand recognized by Toll-like receptor 2 (TLR-2) and Toll-like receptor 4 (TLR-4) on cell surfaces, activating inhibitor of nuclear factor- B (I B) kinase and inducing production of inflammatory cytokines including tumor necrosis factor-α (TNFα), interleukin (IL)-1β, IL-6, IL-8, and chemokines through myeloid differentiation factor 88 (MyD88)-dependent signaling pathways. The resulting high inflammatory cytokine state in the mammary gland and throughout the body activates nuclear factor- B (NF- B). Under normal conditions, NF- B exists in cells as an inactive trimer composed of inhibitor I B bound to NF- B dimers. During inflammation, I B kinase is activated and phosphorylates serine residues on I B, leading to its ubiquitination and degradation by the 26S proteasome. This transforms NF- B from an inactive trimer to an active dimer, thereby upregulating its DNA-binding activity.

Suppressor of cytokine signaling (SOCS) proteins are molecules that can be induced by various cytokines and negatively regulate cytokine signaling pathways. The SOCS family was first discovered in 1997 by Endo et al., and to date, eight members have been identified (CIS, SOCS1, SOCS2, SOCS3, SOCS4, SOCS5, SOCS6, SOCS7). Structurally, all SOCS family members contain a conserved SOCS box at the C-terminus and an SH2 domain in the middle, while the N-

terminus shows considerable variation. Under normal conditions, intracellular SOCS expression levels are extremely low. However, during inflammation, excessive inflammatory cytokines activate the JAK-STAT signaling pathway, and STAT proteins can regulate SOCS expression. The promoter regions of SOCS genes contain STAT-binding sequences, and activated JAK-STAT pathways induce SOCS gene expression, triggering rapid synthesis of SOCS molecules in macrophages and liver cells within 15-20 minutes. The resulting SOCS proteins can inhibit cytokine-mediated JAK-STAT and NF- B signal transduction, forming a negative feedback loop that regulates inflammatory responses.

4. Signal Transduction Mechanism of Milk Protein Content Reduction During Subclinical Mastitis

Milk protein gene transcription is primarily regulated by STAT5, which serves as a marker transcription factor reflecting milk protein gene transcription levels. When dairy cows develop subclinical mastitis, pathogenic bacteria are recognized by pattern recognition receptors on mammary epithelial cells and macrophages—specifically Toll-like receptors—placing the body in a high inflammatory cytokine state. Excessive inflammatory cytokines induce macrophages and liver cells to synthesize SOCS molecules, which negatively regulate cytokine-mediated JAK-STAT signaling pathways. SOCS family members can inhibit JAK-STAT pathways through multiple mechanisms, including suppressing JAK activity, blocking STAT access to receptor binding sites, and promoting proteasomal degradation of signaling proteins. STAT5 must translocate to the nucleus to regulate milk protein gene transcription. Inhibition of the JAK-STAT pathway reduces STAT5 phosphorylation, preventing STAT5 from dissociating from receptors and entering the nucleus. This blocks signal transduction from extracellular to intracellular compartments, preventing STAT5 from binding to STAT5 sites on milk protein gene promoters in the nucleus and initiating gene transcription, thereby suppressing milk protein gene transcription and reducing milk protein yield. Huang et al. found that SOCS3 can negatively regulate the JAK2-STAT5 pathway and inhibit β-casein gene expression in bovine mammary epithelial cells, while SOCS3 knockdown can upregulate milk protein yield. These findings indicate that SOCS-mediated inhibition of JAK-STAT signaling is a key mechanism underlying reduced milk protein content in cows with subclinical mastitis.

During subclinical mastitis, the high inflammatory cytokine state activates the NF- B signaling pathway. Previous research considered NF- B signaling primarily important in immune regulation, inflammatory responses, and tumorigenesis. However, recent studies have revealed that NF- B signaling also plays significant roles in energy balance and metabolic regulation. In vitro studies have shown that NF- B pathway activation can inhibit the PI3K-AKT-mTOR pathway in human glioma U87MG cells. Additionally, skeletal muscle atrophy and reduced protein synthesis in cachexia patients are associated with NF- B-mediated inhibition of the PI3K-AKT-mTOR pathway. Since milk protein gene translation is

primarily regulated by the PI3K-AKT-mTOR pathway, these findings suggest that crosstalk between NF- B and PI3K-AKT-mTOR pathways may be a key mechanism linking mammary infection to reduced milk protein content, though this hypothesis requires further investigation.

In summary, subclinical mastitis alters inflammatory signaling pathways in dairy cows, affecting lactation cell signal transduction and suppressing both transcription and translation of milk protein genes, thereby reducing milk protein content. Therefore, in-depth investigation of the signal transduction mechanisms underlying milk protein reduction during subclinical mastitis will enhance our understanding of the regulatory mechanisms involved and provide important research directions and theoretical foundations for developing better methods to improve milk protein content in dairy cows. This research holds significant importance for improving milk nutritional quality, enhancing the core competitiveness of China' s dairy industry, and ensuring its sustained healthy development.

We thank Professor Sun Dongbo from the College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, for valuable comments on the manuscript.

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