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Comparative proteomics of milk fat globule membrane in goat colostrum and mature milk

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ABSTRACT

As an important nutrient source in large area of world, the composition and nutritional value of goat milk are not well deliberated. Detailed annotation of protein composition is essential to address the physiological and nutritional value of goat milk. In the present study, 423 colostrum and mature goat milk fat globule membrane (MFGM) proteins were identified. The abundance of 189 proteins was significantly different between colostrums and mature milk MFGM. The acute phase proteins were higher in colostrums MFGM than those in mature milk MFGM which protected newborns at the beginning of life. Proteins related to synthesis and secretion were conserved through lactation to ensure the milk production. Of note, long term depression (LTD) proteins were observed in colostrum and mature milk MFGM. Milk LTD proteins could be potential biomarkers for diagnosis of lactation related depressive syndromes and should be taken into considerations of their effects on newborns.

1. Introduction

Goat milk is one of the most important nutritional foods in large area of world, including Asia, Africa and European countries. As an alternative milk source and a potential substitute of human milk in infant formula, goat milk is especially suitable for infants who are allergic to bovine milk (Selvaggi, Laudadio, Dario, & Tufarelli, 2014). However, unlike bovine milk, the comprehensive analysis of goat milk composition is not well achieved. The worldwide increase of production and consumption of goat milk renders the exploration of its nutritional significance.

As the development of proteomics techniques, large number of proteins has been identified and quantified in milk of different species, especially bovine and human milk. In bovine, the differences of milk proteins under various physiological conditions were extensively investigated (Reinhardt, Sacco, Nonnecke, & Lippolis, 2013; Zhang et al., 2015). The proteome of human milk through lactation was also well deliberated by Liao, Alvarado, Phinney, and Lonnerdal (2011). Unlike human and cow milk, the proteome analysis of goat milk is not fully developed during the past few years. The proteome profile exhibited different characteristics among human, cow, yak and goat milk. The protein composition of goat milk is significantly different from that of cow and yak, even they are all ruminants (Lu et al., 2016). Thus, it is not feasible that the knowledge of bovine milk both in lactation and nutrition could be directly applied to explain the significance of goat milk.

Milk fat globule membrane (MFGM), a 3-layer membrane composed of proteins and phospholipids, covers on milk fat globule. MFGM proteins account for 1–4% of milk proteins (Reinhardt & Lippolis, 2006). Proteins with various functions have been identified in MFGM (Lu, van Hooijdonk, Boeren, Vervoort, & Hettinga, 2014; Lu et al., 2011). Bovine MFGM has been used as the nutraceutical materials in dairy industry (Dewettinck et al., 2008). The MFGM is mainly originated from endoplasmic reticulum membrane and plasma membrane with cytoplasm debris trapped in between. Thus, MFGM has been considered as the representative of epithelial cells of mammary gland in studying lactation biology (Cebo, 2012). In present study, MFGM proteins were studied in goat colostrum and mature milk by proteomics analysis. The variations in goat MFGM proteins of colostrum and mature milk could provide us clues to unravel the differences in nutritional value of milk and physiological states of goat during early and middle stage of lactation.

2. Materials and methods

2.1. Sample collection

Colostrum (<7 days postpartum) and mature milk (5 months postpartum) of five Guanzhong dairy goat were obtained in the farm of Shengtang Dairy Co. Ltd (Shanxi Province, China).

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2.2. Separation of MFGM proteins

The separation of MFGM proteins was based on the paper of Lu et al. (2013). In brief, 40 ml milk samples were centrifuged at 1500*g* for 10 min at 10 °C. The cream of centrifuged samples was transferred to a new tube and washed with 40 ml 0.1 M PBS (pH 6.8) and subsequently centrifuged at 1500*g* for 10 min at 10 °C. These steps were repeated for 4 times. Finally, the washed cream was diluted with 0.4% SDS (1:1, v/v), sonicated for 1 min and centrifuged at 1500*g* for 10 min at 10 °C. The cream was discharged and the MFGM proteins were in the aqueous phase. The protein concentration was determined by using BCA assay (Thermo Scientific Pierce BCA protein assay kit, USA).

2.3. Protein digestion

The protein digestion procedure was described before (Lu et al., 2016): 10 μl proteins (1 μg/μl) were diluted with 100 μl 0.05 M NH_4HCO_3. Then 10 μl 0.1 M dithiothreitol was added to the samples and the samples were kept for 30 min at 56 °C. Subsequently, 15 μl 0.5 M iodoacetamide was added to the samples and the samples were kept for 30 min at room temperature in the dark. Trypsin was then added to the samples at a mass ratio of 1:100 enzyme/protein and the samples were kept overnight at 37 °C. The reaction was stopped by adding 1% formic acid. The samples were then desalted by using home-made C18 column.

2.4. EASY-nLC-orbitrap LTQ VELOS

The settings of LC–MS were based on the paper of Ding et al. (2013) and Lu et al. (2016). Digested MFGM protein were injected into a C18 pre-column (100 μm inner diameter, 360 μm outer diameter × 2 cm, 5 μm 150 Å pore size, Durashell C18 particle) and followed by a C18 analytical column (75 μm inner-diameter, 360 μm outer-diameter × 15 cm, 3 μm 150 Å pore size, Durashell C18). The mobile phase of LC was composed of A (0.2% formic acid in water) and B (0.2% formic acid in acetonitrile) and was eluted at flow rate of 380 nl/min with linear gradients. The mass spectrometry was set as followings: The source was operated at 2.0 kV. The MS was programmed in a data dependent acquisition mode. The survey scan was from m/z 375 to 1300 with resolution 60,000 at m/z 400. The 25 most intense peaks with charge state $\geqslant 2$ were acquired in the LTQ normal scan mode.

2.5. ELISA assay

MFGM proteins were separated as described above. The protein concentration of different samples was measured using BCA assay (Thermo Scientific Pierce BCA protein assay kit, USA) and diluted to 1 mg/ml. The ELISA kits for XDH (Cat. No. SEC608Bo), C3 (Cat. No. SEA861Bo), LBP (Cat. No. SEB406Bo) and FN1 (Cat. No. SEA037Bo) were obtained from USCN Business Bio (Wuhan, China). The abundance of the XDH, C3, LBP and FN1 in different samples was measured according to the manufacture's instruction.

2.6. Data analysis

LC–MS/MS raw files were analyzed by using Maxquant 1.5.0.12. The Capra genus proteome database were downloaded from NCBI (http://www.ncbi.nlm.nih.gov/) with reverse database generated by Maxquant. The contaminant database of Maxquant was used. Carbamidomethylation of cysteine was set as fixed modification and oxidation of methionine, N-terminal acetylation, and deamidation of asparagines or glutamine were set as variable modifications. Mass tolerance was set as 20 ppm for MS peaks and 0.5 Da for MS/MS peaks. The FDR was set as 1% and at least 1 peptide was required for identification and quantification of proteins. The abundance of each protein was calculated as followings: protein intensity/summed all identified protein intensity × 100%.

2.7. Statistical analysis

Paired student t-test was applied to compare the protein abundance in colostrum and mature milk with PASW statistics 18 ($P < 0.05$) (SPSS Inc., USA).

2.8. Cluster analysis and Gene Ontology enrichment analysis

Clusters of proteins were analyzed in MultiExperiment Viewer (MeV) package 31 (http://www.tm4.org/mev/). The Gene Ontology (GO) enrichment of proteins was analyzed by using DAVID Bioinformatics Resources 6.7 (http://david.abcc.ncifcrf.gov/) (Huang, Sherman, & Lempicki, 2009).

3. Results

3.1. Identification of MFGM proteins

In total, 423 proteins were identified in MFGM of colostrum and mature goat milk (Supplementary Table 1). Previously observed major MFGM proteins, such as butyrophilin, xanthine dehydrogenase/oxidase, perilipin and milk fat globule EGF factor 8 protein, were all identified in high abundance in present study, which was an indicative of the robustness of the methodology. And 189 proteins were shown to be significantly different between colostrum and mature milk.

3.2. Gene Ontology and KEGG pathway analysis

Most of proteins identified in present study were involved in protein transport and translation. Signal transduction proteins and vesicle mediate transport proteins also accounted for large proportion (Fig. 1a). As expected, MFGM proteins mainly originated from plasma membrane (59 proteins), cytosol (32 proteins) as well as cytoplasmic vesicle (31 proteins). In addition, secreted proteins (43 proteins), mitochondrial proteins (24 proteins) and cytoskeleton proteins (20 proteins) were observed in goat MFGM. Seventy-seven proteins were shown to be involved in the nucleotide binding (Supplementary Table 2). The nucleotide binding proteins identified in the current study are mainly GTP/GDP binding proteins, several members of Ras super family, such as Rabs, Rhos and Rals were identified in milk MFGM. All these proteins are involved in cell skeleton movements as well as secretion of membrane vehicles (Lu et al., 2014; Vetter, Wang, Lorentzen, & Deretic, 2015; Yang et al., 2016). Thus, it is likely these proteins involved in the secretion of vesicle secreted milk components (Lu et al., 2014). Besides nucleotide binding, the other important functions of MFGM proteins were ion binding (49 proteins), lipid binding (19 proteins) and the structural components of ribosome (27 proteins). In present study, 167 out of 423 identified proteins were subjected to KEGG pathways (Fig. 1b). Most of proteins were ribosomal proteins, and cell skeleton related proteins. Large number of proteins was involved in the inflammatory process (chemokine signaling pathway, leukocyte migration, antigen processing and presentation and complement and coagulation cascades). Of note, proteins involved in complement and coagulation cascades, adherens junction and galactose metabolism were only observed in colostrum. Unexpectedly, long term depression proteins (Table 1, Supplementary Fig. 1) were also found in MFGM of colostrum and mature goat milk.