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Research Article

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"Cow's whey Proteins Involved in Mammary Gland Development and Milk Components Synthesis"

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Abstract

Milk is a complex body fluid produced in the mammalian mammary gland. Whey is one of the milk fractions, rich in functional proteins associated with neonate development and lactation. The composition of whey is different by species. Proteomics approaches allowed the identification of thousands of cow's whey proteins. In the present paper we collected data about proteins in bovine whey and using String bioinformatic we divided proteins according to their role in lactation in a cow (mammogenesis and involution; transport and metabolism of various nutrients; cell processes).

Keywords: *Proteomics; Mammogenesis; Involution; Lactation; Whey*

Introduction

Milk is a rich biological fluid, produced by specialized mammary epithelial cells (MEC) in all mammals. The mature mammary gland of a cow consist of four separate glands with teat and associated ducts which lead to the passage of milk to the outside. The right and left sides of the cow's udder are separated by the medial ligament. The microscopy anatomy of the mammary gland is similar among species. Secretory epithelial cells (SEC) creates alveoli. The secretory epithelial cells include necessary organelles to produce and transport milk components. SEC contains numerous mitochondria, well-developed rough endoplasmic reticulum and Golgi apparatus (Nickerson & Akers, 2011).

In epithelial cells, the secretion of milk components into the lumen of lactating alveoli occurs via five different pathways: the exocytosis pathway, the lipid secretion pathway, the transcytosis pathway, carrier-mediated transport across the cell membrane, and extracellular transport (McManaman & Nevile 2003). The proteins of milk have four principal sources among others from blood plasma ("leaky junctions") and due to the activity of mammary gland secretory cells (Fox & Kelly, 2006).

We can distinguish the following milk proteins: caseins, whey proteins, milk fat globule membrane proteins and exomes. In the current paper, we focused on cow's whey proteins and their role in lactation. Milk proteomics tasks are to investigate high-, medium- and low-abundant proteins, describing differences in milk proteome between species, describing changes during transition colostrum into milk and determining biomarkers of mastitis. The most common proteomics approaches in milk studies are electrophoresis 2-D, liquid chromatography coupled via mass spectrometry.

In the current review paper, we collected available data about the whey proteins of cow's milk. Using the String bioinformatic tool whey proteins were divided into categories: proteins associated with mammogenesis and involution, proteins involving in transport and metabolism of various nutrients, and proteins participating in cellular processes (Table 1).

Proteins involved in mammary gland development and involution

Bovine milk contains proteins involved in mammogenesis and mammary gland involution, among others osteopontin (SPP1), matrix metalloproteinases (MMP), lactoferrin (LTF), complement C3, IGLL1 protein, perilipin 2 (PLIN2), fatty acid-binding protein 3 (FABP3), alpha-lactoalbumin (LALBA) and butyrophilin subfamily 1 member A1 (BTN1A1).

FUNCTION		PROTEIN NAME (GENE NAME)
MAMMARY GLAND MAMMOGENESIS AND INVOLUTION		Osteopontin (SPP1); Matrix metalloproteinases 2, 9 (MMP2, MMP9); Beta-1,4-galactosyl-trasferin (B4GALT1); Kappa-casein (CSN3); Beta casein (CSN2); Complement factor 3 (C3); Immunoglobulin lambda (IGLL1); Lactotransferrin (LTF), Fatty acid-binding protein (FABP3); Alpha-lactalbumin (LALBA); Butyrophilin subfamily 1 member A1 (BTN1A1); Perilipin-2 (PLIN2)
METABOLISM AND TRANSPORT OF COMPONENTS	LIPIDS	Apolipoprotein E, A1, A2 (APOE, APOA1, APOA2); Alpha-S1-casein (CSN1S1); Perilipin-2 (PLIN2); Superoxide dismutase (SOD1); Acyl-CoA-binding protein (DBI); Lipoprotein lipase (LPL); CD81 ANTIGEN (CD81); Albumin (ALB); Amyloid beta A4 protein (APP); Epididymal secretory protein E1 (NPC2); MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF); Butyrophilin subfamily 1 member A1 (BTN1A1); Transthyretin (TTR); Proactivator polypeptide (PSAP); Lipopolysaccharide-binding protein (LBP); Fatty acid-binding protein 3, 5 (FABP3, FABP5); Alpha-2-hs-glycoprotein (AHSG)
	CARBOHY-DRATES	Lysosomal alpha-mannosiadase (MAN2B1); L-lactate dehydrogenase B chain (LDHB); Transaldolase (TALDO1); Glucose-6-Phosphate Isomerase (GPI); Vimentin (TPI1); Alpha-enolase (ENO1); Chitinase 3-like 1 (CHI3L1); Malate dehydrogenase (MDH1); Alpha-lactalbumin (LALBA); Peptidoglycan recognition protein (PGLYRP1); Nucleoside diphosphate kinase B (NME1); Glucosidase 2 Subunit Beta (PRKCSH); Beta-1,4-galactosyltrasferin (B4GALT1); Glypican 1, 3, 4 (GPC1, GPC3, GPC4)
	PROTEINS	Dipeptidyl-peptidase 1 (CTSC); Ubiqutin (UBA52); Mannose-binding lectin (MBL2); Cathepsin Z (CTSZ); Complement factor H (CFH); Complement component 1, s subcomponent (C1S); Complement component 2, 3, 5, 6, 7, 9 (C2, C3, C5, C6, C7, C9); Complement C1q protein (C1QB, C1QA); Complement factor H (CFH); Complement component 4A (C4A); Complement factor B, D (CFB, CFD); 72 kDa type IV collagenase (MMP2); Transforming growth factor beta-2 (TGFB2); Plasminogen (PLG); Calreticulin (CALR); Heat shock protein HSP90-ALPHA (HSP90AA1); Heat shock cognate 71kDa protein (HSPA8); FKBP-TYPE peptidyl-propyl cis-trans isomerase (FKBP1A); Glucosidase 2 Subunit Beta (PRKCSH); Protein disulfide-isomerase (P4HB); Peroxiredoxin-4 (PRDX4); Beta-1,4-galactosyltrasferin (B4GALT); Superoxide dismutase (SOD1); Apolipoprotein A2, E, A4 (APOA2, APOE, APOA4); Glypican 1-4 (GPC1-GPC4); Albumin (ALB); Amyloid beta (A4) protein (APP); Beta-2-microglobulin (B2M); Lactotransferrin (LTF); Hemopexin (HPX), Macrophage Migration Inhibitory Factor (MIF); Ephrin-A1 (EFNA1); Tetranectin (CLEC3B); Growth/differentiation factor 8 (MSTN); Chitinase 3-like 1 (CHI3L1); 10kDa heat shock protein (HSPE1); Fibronectin (FN1); Protein deglycase DJ-1 (PARK7); Clusterin (CLU); Pigment epithelium-derived factor (SERPINF1); Thrombospondin-4 (THBS4); Proactivator polypeptide (PSAP); GDP dissociation inhibitor alpha (GDI1); 14-3-3 protein beta/alpha (YWHAB); Carboxypeptidase B2 (CPB2)
	IONS	Coronin-1A (CORO1A); Cathepsin S (CTSS); Prothrombin (F2); Protein deglycase DJ-1 (PARK7); FKBP-TYPE peptidyl-propyl cis-trans isomerase (FKBP1A); Apolipoprotein E, A1 (APOE, APOA1); 14-3-3 protein epsolon (YWHAE); 14-3-3 protein beta/alpha (YWHAB); Macrophage Migration Inhibitory Factor (MIF); Transforming growth factor beta-2 (TGFB2); DNAJ homologue subfamily C member 3 (DNAJC3); Chitinase 3-like 1 (CHI3L); Superoxide dismutase (SOD1); Amyloid beta (A4) protein (APP); Alpha-2-antiplasmin (SERPINF2); Thrombospondin-4 (THBS4); Lactotransferrin (LTF); CD81 Antigen (CD81); Proactivator polypeptide (PSAP); Hemopexin (HPX); Beta-2-microglobulin (B2M); Kininogen-1 (KNG1); Xanthine dehydrogenase/oxidase (XDH)
	VITAMINS	Transcobalamin-2 (TCN2); Vitamin D-binding protein (GC)

PROTEIN SYNTHESIS	INFLUENCE ON GENE EXPRESSION	Macrophage Migration Inhibitory Factor (MIF); Follistatin (FST); Growth/differentiation factor 8 (MSTN); Tetranectin (CLEC3B); Transforming growth factor beta-2 (TGFB2); Lumican (LUM); Ephrin-A1 (EFNA1); High-mobility group box 1, 2 (HMGB1, HMGB2); Profilin-1 (PFN1); Alpha-actin-1 (ACTA1); Vitamin K-dependent protein C (VIM); Protein deglycase DJ-1 (PARK7); Alpha-2-macroglobulin (A2M); Histone H1.2 (HIST1H1C); Histone H2A (H2AFX); Heat shock cognate 71kDa protein (HSPA8), 14-3-3 protein beta/alpha (YWHAB); 14-3-3 protein epsilon (YWHAE); 14-3-3 protein zeta/delta (YWHAZ)
	POST-TRANSLATIONAL MODIFICATIONS	Macrophage Migration Inhibitory Factor (MIF); Transforming growth factor beta-2 (TGFB2); Chitinase 3-like 1 (CHI3L1); DNAJ homologue subfamily C member 3 (DNAJC3); 14-3-3 protein beta/alpha (YWHAB); 14-3-3 protein epsilon (YWHAE); 14-3-3 protein zeta/delta (YWHAZ) Clusterin (CLU)

Table 1: *Proteins involved in mammary gland development, metabolism and transport of various components and protein synthesis.*

During maturation, lactation and involution, the morphological structure of the cow's mammary gland is significantly changed. Mammogenesis takes place in two stages: initially, lactic ducts are elongated and branched, and then under the influence of sex hormones secreted during pregnancy, there is development and maturation of milk lobules and follicles (Rabot et al., 2007). The abovementioned elements constitute the basic structural unit of the mature mammary gland. In addition, the gland is also distinguished by the framework formed by extracellular matrix (EMC) and interstitial tissue (Hu, Li, & Xu, 2017).

Extracellular matrix proteins include osteopontin (SPP1), which is a glycophosphoprotein containing an arginine-glycine-aspartic acid (RGD) cell-binding sequence. The RGD sequence allows the interaction of osteopontins with specific integrins (αVβ3, αVβ5, αVβ1) by mediating in many cellular processes, including initiation of cell adhesion and cell signaling. With the subsequent lactation days, an increase in the concentration of this protein in mammary epithelial cells is observed (Nemir et al., 2000, Zhang et al., 2015a, Zhang et al., 2015b).

The lack or insufficient amount of osteopontin in the mammary gland causes abnormal morphogenesis, which may be manifested in the fact that the milk follicles do not show typical structure for a healthy, mature mammary gland. Moreover, the lack of SPP1 significantly reduces the synthesis of β-casein, whey acidic milk proteins, and also reduces the amount of milk produced, resulting in a lack of food necessary to feed the offspring (Nemir et al., 2000).

Physiological reconstruction of the mammary gland requires controlled EMC degradation and reorganization. Enzymatic proteins are involved in these processes, among others, matrix metalloproteinases (MMP) (Rabot et al., 2007). The presence of MMP9, MMP2 has been demonstrated in cow's milk. MMPs are secreted in the form of proenzymes and are activated in an extracellular environment by a variety of factors, including members of the plasminogen activator (PA) system (Rabot et al., 2007).

Rabot et al. (2007) conducted research to determine the expression of MMPs proteins depending on the developmental state of the mammary gland (pre-lactation maturing, during lactation and during involution) in cattle. These studies have shown that the expression of MMP2 protein increases during mammogenesis. In addition, the authors showed a positive correlation between the increase in the expression of MMPs and the high concentration of plasminogen activator system members. Many studies indicate that matrix metalloproteinase 2 protein is closely related to a branching of lactic ducts in the early stages of mammary gland development (Wiseman et al., 2003; Rabot et al., 2007).

Involution is a physiological process involving the return of the mammary gland to the pre-lactation state (Hurley, 1987; Piantoni et al., 2010). At the tissue level, the involution process is associated with leukocyte invasion, connective tissue cell proliferation and apoptosis or autophagy of mammary epithelial cells (Gajewska et al., 2005; Piantoni et al., 2010).

The largest expression in mammary gland tissue characterized genes related to the response to oxidative stress (Singh et al., 2008) and protein lactoferrin (Zhang et al. 2015b). Lactoferrin is closely related to the involution process. This protein reduces the viability of epithelial cells that form the mammary gland and also inhibits the synthesis of caseins. Furthermore, due to its anti-bacterial role, lactoferrin can protect the mammary gland from infections in the early stages of involution (Riley et al., 2008; Zhang et al., 2015b).

During the reorganization of the mammary gland to the post-lactation state, it becomes susceptible to intra-mammary infections (Dingwell, 2003). It has been shown that during the "preparation" of the mammary gland to involution, the concentration of proteins associated with the immune response increases, including complement C3, IGLL1 protein, osteopontin (Zhang et al., 2015b). The task of C3 and IGLL1 protein is to protect the mammary gland from infections, while the SPP1 protein acts as a chemotactic factor that drives macrophages to inflammatory sites (O'Brien et al., 2011, Zhang et al. 2015b).

During the involution process, the synthesis of milk components is inhibited, moreover, secretory cells of the mammary gland lose their activity (Piantoni et al., 2010, Pai & Horseman, 2011). In the late lactation phase, the expression of milk proteins such as perilipin 2, fatty acid-binding protein 3, alpha-lactoalbumin and butyrophilin subfamily 1 member A1, involved in the milk fat synthesis, is reduced, which is closely related to the beginning of the involution process in the bovine mammary gland (Zhang et al., 2015b).

A number of proteins involved in various cellular processes have been identified in cow's milk, including in cell differentiation, proliferation and migration processes.

Progranulin (PSAP) is a high molecular weight growth factor belonging to the family of granulin proteins (grns) (Desmarais et al., 2008). This protein is involved in the initiation of cell proliferation and migration, particularly epithelial tissue cells (Toh et al., 2011). In addition, PSAP is involved in the development of blood vessels and placenta (Daniel et al., 2003; Desmarais et al., 2008; Toh et al., 2013).

Osteonectin also knows as secreted protein acidic and rich in cysteine (SPARC) is a multi-functional phosphoprotein. This protein is involved in cell proliferation and angiogenesis (Kelleher et al., 2004). Osteonectin has been shown to stimulate cell proliferation and inhibit cell retention in G1 phase of the cell cycle (Burr et al., 2015).

Metabolism and transport milk components Cow's milk contains proteins associated with the metabolism and transport of nutrients, i.e. lipids, proteins, carbohydrates as well as vitamins and ions. The occurrence of enzyme and transport proteins in colostrum and milk determines the proper digestion of nutrients, and also accelerates its absorption, supporting the action of the immature digestive system of newborns.

Enzyme proteins found in cow's milk include many proteinases and peptidases, including dipeptidyl-peptidase (CTSC), carboxypeptidase (CPB2), cathepsin S (CTSS) and cathepsin Z (CTSZ). Most milk proteinases belong to the cathepsins cysteine protein family that are activated at the acidic pH present in the stomach (Turk et al., 2012). Too high pH-value in the stomach of calves prevents from hydrolysis of cow's milk proteins. The provision of suitable proteinases with mother's milk determines the protein degradation and the release of bioactive peptides in the stomach of young individuals (Le et al., 2011).

One of the proteins found in colostrum and milk of cows is fatty acid binging protein (FABP3). FABP3 is responsible for the transport of endothelial long-chain fatty acid to the endoplasmic reticulum, where triglyceride synthesis takes place, which are then redistributed in the form of drops of fats within the cell (Bionaz & Loor, 2008). In addition, among proteins involved in the metabolism and transport of lipids, we can distinguish, among others, butyrophilin subfamily 1 member A1 (BTN1A1) and perilipin-2 (PLIN2), that are involved in the synthesis of milk fat (Bionaz & Loor, 2008; Zhang et al., 2015a). Expression of FABP3, BTN1A1 and PLIN2 increases with subsequent lactation days, which according to Zhang et al. (2015a), indicates that these proteins are involved in the de novo mammary fatty acid synthesis.

Proteins characterized by decreasing expression in bovine milk with subsequent lactation days include apolipoproteins family ones (APOA1, APOA4, APOE) (Zhang et al., 2015a; Zhang et al., 2015b). APOs play a key role in lipid transport, synthesis, and catabolism of plasma lipoproteins, and are involved in the activation of many enzymes associated with fat metabolism (Donma & Donma, 1989). APOE is a cholesterol transporter, which through its participation in the synthesis of vitamin D and steroid hormones, determines the proper development of newborns. Zhang et al. (2015b) showed a high concentration of this protein in cow's milk in the initial phase of lactation.

Serotransferrin (TF), selenium binding protein 1 (SELENBP1), vitamin D-binding proteins (GC) are proteins involved in the transport of ions and vitamins. Expression of these proteins in bovine milk decreases with subsequent lactation days (Zhang et al., 2015a). Vitamin D-binding protein, also know as GC-globulin, is a vitamin D-binding protein, as well as fatty acids and actin monomers, preventing their polymerization. The GC protein transports vitamin D to various cells (tissues), regulating its total amount within the

body (Chun, 2012). Vitamin D can also be bound to albumin, the expression of which, like GC proteins, decreases with subsequent days of lactation (Chun, 2012; Zhang et al., 2016). Vitamin D plays a key role in many processes, including bone mineral metabolism, proper functioning of cells and maintenance of calcium homeostasis. As demonstrated by Hossain et al. (2014), a high concentration of vitamin D-binding protein (GC) in the initial phase of lactation is important in the management of vitamin D in newborns.

Serotransferrin and selenium binding protein 1 are involved in ion transport. TF delivers iron to cells via receptor-mediated basolateral endocytotic process providing antimicrobial activity. SELENBP1 is involved in the intercellular transport of selenium, which acts as a cofactor reducing antioxidant enzymes, including glutathione peroxidases. According to Zhang et al. (2016), decreasing expression of these proteins along with the progress of lactation may indicate that iron and selenium are essential elements in the early development of suckling newborns.

Proteins involved in protein synthesis

Among milk proteins, such ones influencing the processes regulating the gene expression and post-translational modification of proteins can also be distinguished (Table 1). In bovine milk, proteins belonging to the 14-3-3 protein family have been identified (Le et al., 2011; Tacoma et al., 2016). Proteins belonging to this family are activators of neuromediator synthesis, interact with many enzymes, structural proteins and cytoskeletal proteins, proteins involved in the cell cycle and control of transcription, as well as also participate in apoptosis (Yaffe, 2002). There are seven isoforms of these proteins in animals (β, ε, η, γ, τ, ζ and σ) (Obšilová et al., 2008), of which five isoforms were identified in bovine milk: 14-3-3 protein beta/alpha (YWHAB), 14-3-3 protein zeta/delta (YWHAZ) and 14-3-3 protein epsilon (YWHAE), that were present only in colostrum (Yaffe, 2002; Le et al., 2011; Tacoma et al., 2016).

Myostatin (MSTN) is a protein belonging to the transforming growth factor β (TGF-β), and its main role is to regulate the processes associated with the growth and differentiation of cells. In addition, TGF-β bind to membrane receptors to form a complex phosphorylating protein from the SMAD family, thereby participating in the post-translational modification of proteins. The SMADs, by binding to DNA, recruit activators or repressors of the transcript that control the expression of genes. The TGF-β is closely related to the follistatin protein (FST), which binds to myostatin and inhibits its attachment to the ActRIIB receptor, indirectly contributing to the regulation of gene expression (Zhu, Topouzis, Liang, & Stotish, 2004).

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