OPINION LEADER

Key AA molecular signalling pathways in the regulation of milk synthesis

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Abstract

Mammary gland is responsible for milk synthesis in mammals, predominantly determining neonatal growth and health. Amino acids are the building blocks for milk protein and energy sources for neonates. Recent advances demonstrated that some functional amino acids also regulate milk protein and fat synthesis through distinct intracellular and extracellular pathways. In this study, we discuss recent advances in the role of amino acids (especially branched chain amino acids, methionine, arginine and lysine) in the regulation of milk synthesis.

Background

Milk is primarily composed of milk protein, fat, lactose, vitamins and minerals, which are important nutrient sources for neonates. Thus, mechanically understanding nutritional strategies to regulate milk synthesis is important for mammals. Amino acids are not only basic components of proteins, but also act as functional regulators in a variety of biological processes. Various amino acids (especially branched-amino acids, methionine and arginine) are involved in the regulation of milk synthesis. Signaling pathways regulating mammary epithelial cell proliferation and differentiation have been well characterized [1].
However, the underlying mechanisms and signaling pathways by which amino acids regulate milk and fat synthesis were largely unknown. The aim of this review is to describe how amino acids functions in the regulation of milk synthesis in the mammary gland.

Branched-chain amino acids

Transportation of BCAAs in the mammary gland

The plasma membrane transport system L is the most critical amino acid transporter system for BCAAs in mammary cells [2]. Transporters from the L system are heterodimeric proteins, which comprise of a catalytic subunit (L-type amino acvid transporter 1 (LAT1), encoded by SLC7A5, or L-type amino acid transporter 2 (LAT2), encoded by SLC7A6 and a glycoprotein 4F2 heavy chain (4F2hc). Both LAT1 and LAT2 are highly expressed in the mammary tissues [2].

Potential signaling pathway of BCAAs in the mammary gland

Leucine

In the mammary gland, leucine regulates various biological processes, such as cell proliferation and milk synthesis (αs-casein, β-casein, and κ-casein) (as shown in Table 1). mTOR functions as a critical regulator of these processes, but it was not clear of how leucine regulates mTOR until recently (Figure 1). Leucine-regulated mTOR signaling pathway is conserved in the mammary gland. In bovine mammary glands, overexpression of Sestrin2 depresses mTORC1 activity and synthesis of casein [3]. Interestingly, not only leucine but also other essential amino acids and nonessential amino acids can also regulate mTORC1 through Sestrin 2 in the mammary gland [4], which warrants further investigation. In the mammary gland, the other potential leucine-mediated mTORC1 signaling pathway is through guanine nucleotide-binding protein subunit gamma-12 (GNG12) and leucyl-tRNA synthetase (LeuRS). GNG12 regulates the mTORC1 via interaction with Regulator [5]. LeuRS acts as a vital intracellular leucine sensor that can directly bind to Rag GTPase and activate mTORC1 [6].

Table 1. Effects of BCAAs on mammary gland function and its potential signaling pathways.

Amino acids	Cell lines/Animal species	Functions	Potential signaling pathways	References
BCAA (-)	Cows mammary gland (in vivo,105 ± 12 d of lactation for 5 d)	Milk yield ↓	Inhibit mTORC1/eIF2Bε /eIF2α signaling pathway	[28]
Leucine (+)	Bovine mammary epithelial cell/Bovine mammary tissue slices	Milk protein synthesis ↑	Activate mTOR/S6K1 signaling pathway	[29]
Leucine (+)	Mouse mammary gland (in vivo, from parturition through d 17 of lactation)	–	Activate Akt/mTOR signaling pathway	[30]
Leucine (+)	Mouse mammary epithelial cell	Proliferation ↑	LAT1 and leucyl-tRNA synthetase	[31]
Leucine (+)	Mice mammary gland (in vivo, from parturition through d 17 of lactation)	β-Casein synthesis ↑	Activate mTOR signaling pathway	[30]
Leucine (+)	Bovine mammary epithelial cells	αs-, β-, κ-casein synthesis ↑	Activate mTOR signaling pathway	[32]
Leucine (+)	Bovine mammary epithelial cells	Expression of casein genes (CSN1S1, 2, 3) ↓	Activate JACK2/STAT5 and mTOR signaling pathway Inhibit AMPK phosphorylation"	[33]
Leucine (-)	Mid-lactation Holstein cows (in vivo, 108 ± 11 d of lactation for 5 d)	Milk protein yield ↓ Synthesis of αS1,β, κ-casein ↓	–	[34]
Leucine (-)	Bovine mammary epithelial cell/Bovine mammary tissue slices	Milk protein synthesis ↓ Protein synthesis rates ↓	Inhibit mTOR/S6K1 signaling pathway	[29]
Isoleucine (+)	Bovine mammary epithelial cell/Bovine mammary tissue slices	Milk protein synthesis ↑	Increase mTOR/S6K1 signaling pathway	[29]
Isoleucine (+)	Mouse mammary gland (in vivo, from parturition through d 17 of lactation)	–	Increase Akt/mTOR signaling pathway	[30]
Isoleucine (-)	Bovine mammary epithelial cell/Bovine mammary tissue slices	Milk protein synthesis ↓ Protein synthesis rates ↓	Inhibit mTOR/S6K1 signaling pathway	[29]
Valine (+)	Porcine mammary epithelial cells	Milk fat synthesis ↑	Akt/mTOR/SREBP1 pathway	[35]
Valine (+)	Porcine mammary epithelial cells	α-Lactalbumin and β-casein synthesis ↑	mTOR and Ras/ERK signaling pathways	[36]

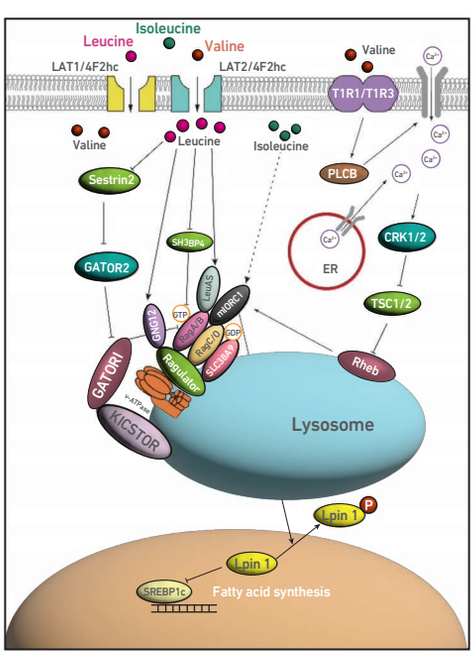

Figure 1. BCAAs and mTORC1 signaling networks in the mammary gland.

LAT1/4F2hc and LAT2/4F2hc derived from transporter system L are highly expressed and play a dominant role in BCAA transportation in the mammary gland. All three BCAAs activate mTORC1 pathways in mammary glands.
In the mammary gland, activated mTORC1 not only increases milk protein synthesis but also milk fat synthesis through lipin 1-SREBP1c pathways. Please refer to the main text for details.

Valine and Isoleucine

Dietary supplementation of L-isoleucine and L-valine during lactation enhances milk synthesis in sows [7]. Both L-isoleucine and L-valine can activate the mTOR signaling pathway and have the potential to enhance milk protein synthesis [8, 9]. As L-valine regulates lipogenesis through activation of mTORC1, L-leucine and L-isoleucine might also participate in the lipogenesis progression. T1R1/T1R3, a G-protein-coupled receptor (GPCR) in the cell membrane, can be widely activated by L-amino acids [10], hence BCAAs could activate mTORC1 through this signaling pathway in the mammary gland.

Methionine

Transportation system of methionine in the mammary gland

In the mammary gland, three amino acid transporter systems are involved in methionine transportation, namely systems A, ASC and L [11]. System A consists of sodium-coupled neutral amino acid transporter 1 (SNAT1) (detected in pigs) and SNAT2 (detected in rats and cows) [12]. System ASC primarily contains Na-dependent alanine cotransport 1 (ASCT1) (detected in humans, mice, cows and pigs) and ASCT2 (detected in rats and cows) [12]. System L is composed of two heteromeric Na+-independent transporters LAT1/4F2hc (detected in humans, rats, mice, cows) and LAT2/4F2hc (detected in rats and cows) [13].

Potential signaling pathway of methionine in the mammary gland

When methionine is deficient, the cellular methyl donor S-adenosylmethionine (SAM) level will be reduced, which further increases the association of SAMTOR (SAM sensor) with GATOR2 and inhibits mTORC1 signaling [14]. Two other potential methionine-regulated mTORC1 signaling pathways have been verified in the mammary gland (Figure 2). One possible approach is that methionine may regulate the milk lipid synthesis through the PI3K/Akt/-FABP5/SREBP1c signaling pathway. The other novel and crucial signaling pathway is that methionine also increases the influx of intracellular Ca2+ and regulates the effects of mTORC1 through T1R1/T1R3 in the mammary gland [15].

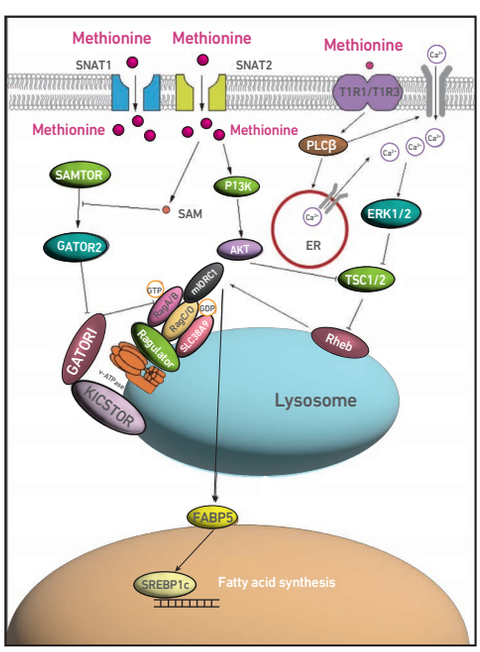

Figure 2. Methionine and mTORC1 signaling networks in the mammary gland.

SNAT1 and SNAT2 originate from transporter system A and are crucial methionine transporters in the mammary gland. Activated mTORC1 increases milk protein synthesis and regulates milk fat synthesis through SREBP1 and FABP5. Please refer to the main text for details.

Arginine and lysine

Transportation system of arginine and lysine in mammary gland

Arginine and lysine are both cationic amino acids and have the same amino acid transporter systems in the mammary gland [16]. Four cationic amino acid transporter systems have been identified in the mammary gland as follows: (1). y+ system: cationic amino acid transporter 1 (CAT-1, detected in humans, cows, pigs and rats) and CAT2 (detected in pigs); (2). y+L system: y+LAT1(detected in pigs and cows) and y+LAT2 (detected in pigs); (3). b0,+ system: b0,+AT (detected in pigs); and (4). B0,+ system: ATB0,+ (detected in pigs, humans, rats) [17, 18].

Potential signaling pathway of arginine and lysine in the mammary gland

Similar to other amino acids, arginine also regulates milk protein synthesis through mTORC1 [19]. The central regulator linking arginine to the mTORC1 signaling pathway is CASTOR1, and sufficient argi nine dissociates GATOR2 from CASTOR1 and further activates the mTOR signaling pathway [20, 21] (Figure 3). The other crucial function of arginine in the mammary gland is primarily achieved through its metabolite nitric oxide (NO) [22]. Briefly, NO increases the mammary blood vessel density and diameter, which might support milk synthesis [23].

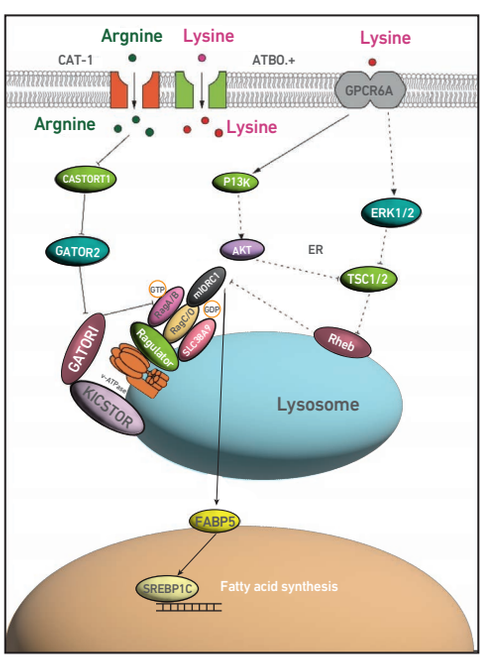

Figure 3. Lysine and arginine regulate the mTORC1 signaling network in the mammary gland.

The intracellular arginine regulator mTORC1 acts through the CASTOR1/GATOR2/GATOR1/RagA/B signaling pathway, whereas extracellular lysine regulates mTORC1 through the GPRC receptor GPCR6A. Please refer to the main text for details.

Similar to methionine, lysine is one of the first two limiting amino acid both in dairy cows and sows. When lysine is deficient, milk protein synthesis is inhibited [24] with a decrease in mTORC1 activity in dairy cows [25]. However, the function of lysine in the mammary gland has largely not been determined. Recent advances have found that lysine increases milk fat synthesis through the GPRC6A-PI3K-FABP5 signaling pathway [26]. As a Gαi/Gαq receptor, GPRC6A has the potential to regulate cellular cAMP levels and activate the MAPK signaling pathway [27]. Thus, both GPRC6A/PI3K/AKT/mTOR and GPRC6A/ERK/mTOR can be the potential signaling pathways for lysine to regulate milk protein and fat synthesis in the mammary gland.

Conclusions

Amino acids play crucial roles in the synthesis of milk protein and fat in the mammary gland. The dominant amino acid transporters (BCAAs, methionine, lysine and arginine) of the mammary gland are summarized in this review. In addition, our review has focused on a number of canonical and novel signaling molecules involved in amino acid signaling pathway in the mammary gland. Remarkably, mTORC1 acts as the central node of the amino acid-regulated signaling pathway and can be activated intracellularly and extracellularly (through - GPCR). Achieving a better understanding of the amino acid signaling pathway might help us to optimize the amino acid profiles in maternal diets for human beings and other mammals in the future.

List of abbreviations

ASCT1: Na-dependent alanine co-transport 1; BCAA: branched chain amino acids; CAT-1: cationic amino acid transporter 1; FABP5: Fatty acid-binding protein 5; GNG12: guanine nucleotide-binding protein subunit gamma-12; GPCR: G-protein-coupled receptor; PI3K: inositol 1,4,5-trisphosphate 3-kinase; LAT1: L-type amino acid transporter 1; LAT2: L-type amino acid transporter 2; LeuRS: leucyl-tRNA synthetase; mTORC1: mammalian target of rapamycin complex 1; SAM: methyl donor S-adenosylmethionine; SH3-BP4: danio rerio SH3-domain binding protein 4; SNAT1: sodium-coupled neutral amino acid transporter 1; SREBP-1: Sterol regulatory element-binding protein 1.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SZ initiated the idea, the scope, and the outline of this review paper. ZW studied and analyzed all of the publications cited in this paper and was involved in the manuscript preparation. WG conducted the final editing and proofreading. All authors read and approved the final manuscript.

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