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RESEARCH HIGHLIGHT

Glutamine-released ammonia acts as an unprecedented signaling molecule activating lipid production

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In healthy humans, ammonia circulates in the bloodstream at a concentration around 11–32 μmol/L. Whether ammonia plays any physiological role has been unknown until a recent report published in *Nature Metabolism*.[1] Ammonia, long thought to be a toxic waste product of amino acid metabolism, needs to be excreted from the human body as urea. Intriguingly, in this paper, Cheng et al[1] for the first time reported that ammonia is not a waste product; rather, it is an unprecedented signaling molecule tying glucose and glutamine to lipid production in human cells. This study revealed that ammonia released from glutamine triggers a cascade of cellular processes leading to the activation of lipogenesis machinery for lipid production (Fig. 1). This discovery has major implications for developing treatments for human diseases such as cancers and metabolic syndromes, as dysregulated metabolism is a hallmark of these diseases.

In eukaryotic cells, lipid synthesis is mainly regulated by sterol regulatory element-binding proteins (SREBPs), a family of transcription factors that contain SREBP-1a, -1c and -2.[2,3] After synthesis, SREBP precursors (~125 kD) bind to the endoplasmic reticulum (ER) membrane in a complex with SREBP cleavage-activating protein (SCAP) and insulin-induced gene protein (Insig), which includes Insig-1 and -2 (ER-resident proteins). Lipids are essential components of cellular membranes, while excessive lipids, such as fatty acids and cholesterol, causes toxicity for cells. Therefore, SREBP transcriptional activity and lipid production rates are tightly controlled. SREBPs first need to exit the ER and translocate to the Golgi apparatus for a sequential cleavage by two proteases (site-1 protease (S1P) and site-2 proteases (S2P)), which release the active N-terminal SREBP forms (~65 kD) that enter into the nucleus to activate lipogenic gene expression.[2,3] The key step controlling SREBPs' exit from the ER is regulated by SCAP, which binds to COPII-coated vesicles to transport SREBPs from the ER to the Golgi. However, this step is restricted by the ER-resident protein Insig, which directly binds to SCAP to prevent it from interacting with COPII proteins, thereby spatially restricting the SCAP/SREBP complex to the ER.[2] Thus, the key step for SREBP activation is SCAP escaping from Insig binding. Prior to 2015, understanding of SCAP/

Abbreviations: SCAP, SREBP-cleavage activating protein; SREBP, Sterol Regulatory Element-Binding Protein; Insig, Insulin-Induced Gene Protein; 25-HC, 25-Hydroxycholesterol; GLS, Glutaminase; S1P/S2P, Site 1 / Site 2 Proteases.

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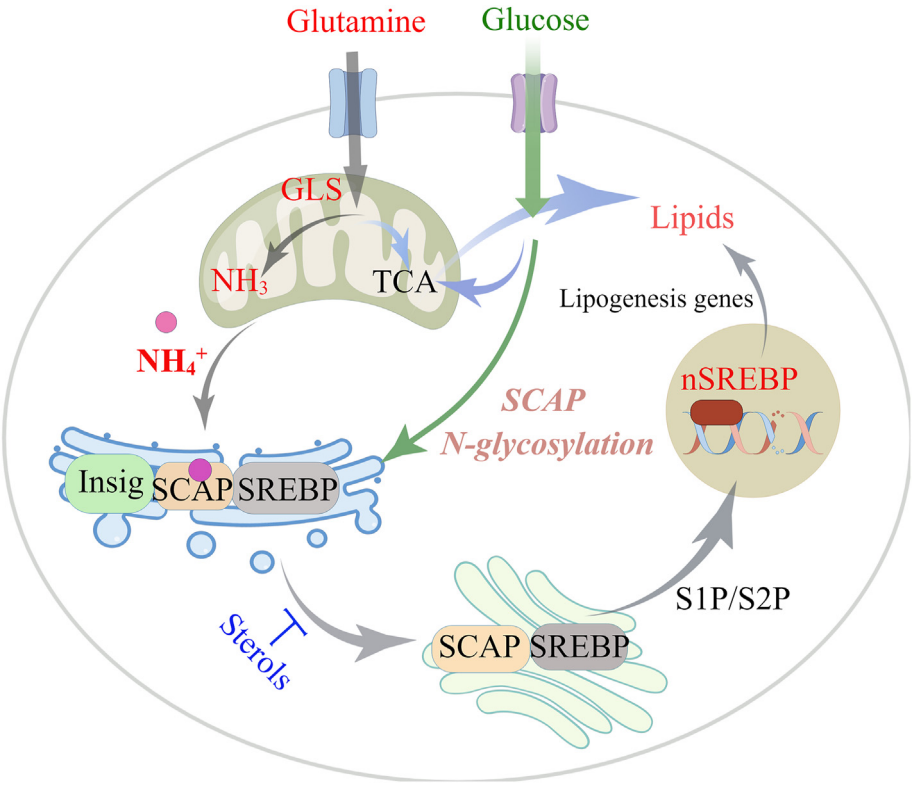

Figure 1 Glutamine-released ammonia is a signaling molecule activating lipid production. Catalyzed by GLS, ammonia (NH_3) released from glutamine converts to NH_4^+ by protonation that acts as a key activator stimulating *N*-glycosylated SCAP dissociation from Insig via its binding to SCAP. It leads to SREBP exit from the ER and translocation to the Golgi apparatus for sequential cleavage by S1P and S2P proteases. This releases the active N-terminal SREBP form (nSREBP) that enters into the nucleus to activate lipogenic gene expression and promote *de novo* lipid synthesis. Both glucose and glutamine are substrates for lipid biosynthesis, each being metabolized to citrate in the TCA cycle, which is exported from the mitochondria to the cytosol to serve as a precursor for lipid production controlled by SREBP-regulated lipogenic genes. Ammonia (NH_4^+) acts as a critical signaling molecule, signifying nutrient sufficiency to lipogenesis machinery by activating SCAP/SREBP translocation, thereby stimulating lipid production. Accumulation of sterols like cholesterol and 25-hydroxycholesterol in the ER membrane decelerates this process by binding to SCAP and Insig to enhance their association and restrain SCAP/SREBP in the ER. The balance between glucose, glutamine and sterols precisely controls lipid synthesis rates. Dysregulation of this balance is found in a diversity of diseases, such as obesity, diabetes, cardiovascular diseases and cancer. GLS, glutaminase; Insig, insulin-inducible gene; S1P, site-1 protease; S2P, site-2-protease; SREBP, sterol regulatory element-binding protein; SCAP, SREBP cleavage-activating protein; TCA cycle, tricarboxylic acid cycle.

Insig dissociation and SREBP translocation remained limited to the classical sterol-mediated negative feedback loop model established by Nobel Laureates Brown and Goldstein in the 2000s. When sterols, including cholesterol and 25-hydroxycholersterol (25-HC), from lipid synthesis build up in the cell, they will bind to SCAP or Insig to enhance the association between Insig and SCAP, thereby inhibiting SREBP translocation and slowing down lipogenesis (Fig. 1). However, whether SCAP dissociation from Insig requires an activator to stimulate has been unknown until a series of recent studies from our group.[1–4]

Early in 2009, we demonstrated that oncogenic EGFR/PI3K/Akt signaling activates SREBP-1 to promote lipogenesis and tumor growth.[3] In 2015, we found that EGFR signaling, via increasing glucose uptake, promotes SCAP N-glycosylation and stability, which is a prerequisite for activating SREBPs.[4,5] When glucose level decreases, SCAP is quickly degraded and SREBP activation is inhibited, which shuts down lipid production to save energy for cells going through metabolic stress. This study suggested that glucose acts as an activator for SREBPs. Unexpectedly, in following studies we found that, when glutamine is absent, even under high level of EGFR signaling, abundant glucose and low sterol conditions, SCAP/Insig dissociation and SREBP activation cannot occur. These results demonstrated that glucose alone is necessary but not sufficient to activate SREBPs, which requires the concurrent presence of glutamine to ultimately activate lipogenesis machinery.[1]

Glutamine concentration is around 700 μmol/L in the human bloodstream, the highest concentration among all 21 amino acids. Glutamine is an unessential amino acid as it can be synthesized from glutamate. However, in certain contexts, such as cancer, it becomes essential as rapid tumor growth consumes large amounts of this amino acid. Glutamine has multiple functions. It is a substrate for protein, nucleotide, and lipid synthesis. It also supports energy production through glutaminolysis.[1] Glutamine is deaminated by glutaminase (GLS), which converts it to

glutamate, releasing one ammonia molecule. Glutamate is further converted to α-ketoglutarate, which enters the tricarboxylic acid (TCA) cycle for energy production. To identify the molecular mechanism underlying the activation of SREBPs by glutamine, we examined which metabolite derived from glutaminolysis plays a role. Surprisingly, we found that ammonia, not glutamate or α-ketoglutarate, is required along with glucose to activate the release of SCAP from Insig and subsequent SCAP/SREBP translocation.[1] By performing a biochemical pulldown assay, we determined that ammonia directly binds to SCAP to trigger its release from Insig. The data strongly suggested that ammonia could be the unknown activator responsible for triggering SCAP/Insig dissociation and lipid synthesis (Fig. 1).

Next, we closely examined SCAP structure by using computational modeling techniques and identified three potential binding sites for ammonia on SCAP.[1] These include an aspartate D428 residue from its sixth transmembrane helix and serine S326/S330 residues from its third transmembrane helix that constitute the central transmembrane domain. Molecular dynamics simulations demonstrated that ammonia forms three stable hydrogen bonds with these residues to tightly bind to SCAP. Ammonia binding stimulates a sequential conformational change of SCAP transmembrane helices, which reduces the contact area between SCAP and Insig, eventually leading to the separation of SCAP from Insig and SREBP translocation. We further found that 25-HC blocks ammonia from accessing its binding sites on SCAP by physically occupying the channel leading to the sites. These findings unveiled the competitive role between ammonia and sterols. The balance of these two opposing forces controls the strength of SCAP/Insig association and therefore lipid synthesis rates (Fig. 1).

In summary, our new study uncovered, for the first time, that the process and control of cellular lipid production is tightly regulated by glucose and glutamine availability (Fig. 1).[1] These results put forth an elegant model for the regulation of SCAP/Insig dissociation and SREBP translocation. Activation of this process requires sufficient glucose and glutamine to be present, while sterols function as a negative regulator as they accumulate in the ER membrane. This balance maintains proper lipid levels in living cells and organs of human body. Dysregulation of this balance results in various diseases such as obesity, fatty liver, cardiovascular and neurological diseases, as well as cancer. From this perspective, limiting excessive nutrient availability and uptake and intervening in ammonia production and its binding with SCAP could offer promising new avenues to treat these diseases.

Conflict of interests

All authors declare no conflict of interests.

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