BMB
Reports

BMB Rep. 2023; 56(7): 385-391
www.bmbreports.org

Contributed Mini Review

Aspartate-glutamate carrier 2 (citrin): a role in glucose and amino acid metabolism in the liver

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Aspartate-glutamate carrier 2 (AGC2, citrin) is a mitochondrial carrier expressed in the liver that transports aspartate from mitochondria into the cytosol in exchange for glutamate. The AGC2 is the main component of the malate-aspartate shuttle (MAS) that ensures indirect transport of NADH produced in the cytosol during glycolysis, lactate oxidation to pyruvate, and ethanol oxidation to acetaldehyde into mitochondria. Through MAS, AGC2 is necessary to maintain intracellular redox balance, mitochondrial respiration, and ATP synthesis. Through elevated cytosolic Ca^{2+} level, the AGC2 is stimulated by catecholamines and glucagon during starvation, exercise, and muscle wasting disorders. In these conditions, AGC2 increases aspartate input to the urea cycle, where aspartate is a source of one of two nitrogen atoms in the urea molecule (the other is ammonia), and a substrate for the synthesis of fumarate that is gradually converted to oxaloacetate, the starting substrate for gluconeogenesis. Furthermore, aspartate is a substrate for the synthesis of asparagine, nucleotides, and proteins. It is concluded that AGC2 plays a fundamental role in the compartmentalization of aspartate and glutamate metabolism and linkage of the reactions of MAS, glycolysis, gluconeogenesis, amino acid catabolism, urea cycle, protein synthesis, and cell proliferation. Targeting of AGC genes may represent a new therapeutic strategy to fight cancer. [BMB Reports 2023; 56(7): 385-391]

INTRODUCTION

Aspartate-glutamate carrier (AGC) is a calcium-dependent mitochondrial carrier that transports aspartate from mitochondria to the cytosol in exchange for glutamate (1-3). The driving force for exchange is the proton gradient created by the respiratory chain of the mitochondria. The Ca^{2+}-dependent stimulation is enabled by the presence of a unique long N-terminal extension containing eight EF-hand motifs facing the intermembrane space for calcium binding (3-5). Therefore, the signals that affect the cytosolic concentration of Ca^{2+}, such as glucagon and catecholamines, which elevate cellular Ca^{2+} levels by stimulation of its release from endoplasmic reticulum and uptake from extracellular fluid (6-8), can modulate the flux of aspartate and glutamate through AGC.

There are two AGC isoforms: AGC1 (aralar 1) and AGC2 (citrin), which are encoded by SLC25A12 and SLC25A13, respectively. AGC1 is found in several tissues, mainly in the heart, skeletal muscle, and brain, whereas AGC2 is most abundantly expressed in the liver and gastrointestinal tract (1-3, 9). Although AGC1 and AGC2 play the same role in the mitochondrial membrane, i.e., ensuring equimolar exchange of glutamate and aspartate between mitochondria and the cytosol, their physiological roles differ, due to the different functions of tissues in which they are found.

The objective of this article is to examine the physiological importance of the compartmentalization of aspartate and glutamate metabolism in the liver, which is provided by AGC2. The focus is on the malate-aspartate shuttle (MAS), urea cycle, gluconeogenesis, and cell proliferation. We are not aware of any previous review article that has examined the involvement of AGC2 in these metabolic pathways, which are of fundamental importance for the position of the liver as a central organ in intermediary metabolism.

AGC2 AND THE COMPARTMENTALIZATION OF ASPARTATE AND GLUTAMATE METABOLISM

The distribution of aspartate and glutamate between mitochondria and the cytosol ensured by AGC2 is of fundamental importance for the compartmentalized metabolism of these amino acids in the liver (Fig. 1).

Glutamate that is synthesized in mitochondria by glutaminase (Gln → NH_3 + Glu), or in the cytosol during amino acid catabolism, and subsequently transported into the mitochondria by AGC2 and other glutamate carriers, such as SLC25A22 and SLC25A18, is used by glutamate dehydrogenase to form ammonia (glutamate → NH_3 + 2-OG), or by aspartate aminotransferase (AST) to form aspartate (glutamate + OA → 2-OG + aspartate). Aspartate synthesized in mitochondria is trans-

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https://doi.org/10.5483/BMBRep.2023-0052

Received 5 April 2023, Revised 19 May 2023, Accepted 29 May 2023, Published online 14 June 2023

Keywords: Gluconeogenesis, Malate-aspartate shuttle, Mitochondria, Oxaloacetate, Urea cycle

ISSN: 1976-670X (electronic edition)

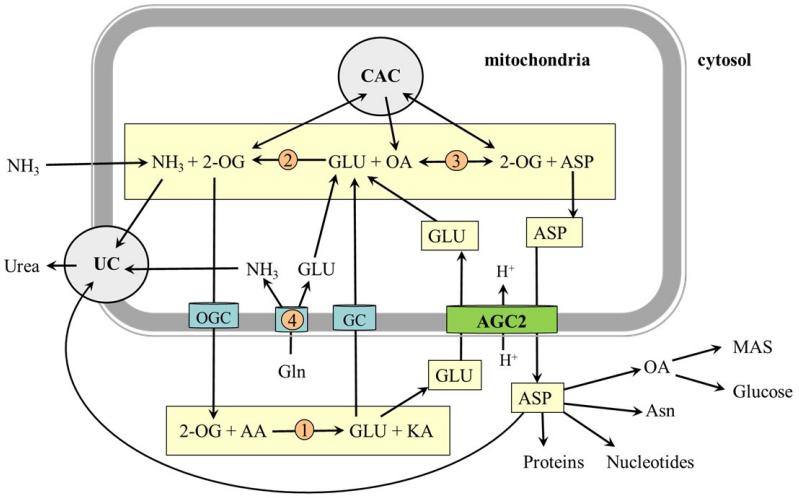

Fig. 1. The role of AGC2 in compartmentalization of aspartate and glutamate metabolism in the liver. Note that 2-OG produced in mitochondria by glutamate dehydrogenase and AST can be via the CAC converted to OA, the initial substrate for gluconeogenesis. 1, Amino acid transferase (2-OG dependent); 2, glutamate dehydrogenase; 3, AST mitochondrial; 4, glutaminase. AA, amino acids; AGC2, aspartate-glutamate carrier 2; ASP, aspartate; CAC, citric acid cycle; GC, glutamate carrier; GLU, glutamate; KA, keto acids; MAS, malate-aspartate shuttle; OGC, 2-oxoglutarate carrier; OA, oxaloacetate; UC, urea cycle; 2-OG, 2-oxoglutarate.

ported to the cytosol by AGC2 (1-3), to be used in the urea cycle or for the synthesis of proteins, asparagine, nucleotides, and oxaloacetate (OA) that can enter MAS or gluconeogenesis reactions. Since mitochondrial AST activity and glutamate supply are very high, aspartate concentrations in hepatocytes are more than a hundredfold higher than in plasma (10, 11).

It should be emphasized that the influence of AGC2 on aspartate and glutamate metabolism in the liver depends on the metabolic zone of the liver lobule (12, 13). Periportal hepatocytes (zone I hepatocytes) are specialized for oxidative metabolism, such as gluconeogenesis, ammonia detoxification to urea, beta-oxidation of fatty acids, and cholesterol synthesis. A much smaller number of perivenous hepatocytes (zone III hepatocytes) are important for glutamine synthesis, glycolysis, lipogenesis, and reactions catalyzed by cytochrome P-450. Unfortunately, it is not known whether there are differences in the expression of AGC2 among individual zones of the lobule.

AGC2 AND THE MALATE-ASPARTATE SHUTTLE (MAS)

The MAS consists of two enzymes, malate dehydrogenase (MDH) and AST, which are found both in the mitochondrial matrix and in the cytosol, and two transporters, AGC2 and 2-oxoglutarate carrier (OGC) that are localized in the inner mitochondrial membrane (14).

The textbooks of biochemistry state that the main function of the MAS is the indirect transfer of NADH produced in the cytosol during glycolysis by 3-phosphoglyceraldehyde dehydrogenase into the mitochondria. Other common sources of NADH in the liver are pyruvate synthesis from lactate by lactate dehydrogenase, and the oxidation of ethanol to acetaldehyde by alcohol dehydrogenase. Therefore, the MAS plays a central role in maintaining intracellular redox balance, mitochondrial respiration, and ATP synthesis.

Fig. 2 shows that the movement of NADH from the cytosol into the mitochondria is linked to the synthesis of OA from aspartate supplied to the cytosol by AGC2. After that, the cytosolic MDH catalyzes the reaction of OA and NADH to produce malate and NAD^+. Malate transported to mitochondria by OGC is converted by mitochondrial MDH, the enzyme of the CAC, to OA. In this reaction, NAD^+ is reduced to form NADH that is funneled into the respiratory chain to create an electrochemical potential across the inner mitochondrial membrane that is used by ATP synthase to produce ATP. OA produced in MDH reaction is used by mitochondrial AST to reconstitute aspartate. In summary, MAS is formed by four enzymatic reactions:

the cytosol:	aspartate + 2-OG → OA + glutamate
	OA + NADH + H^+ → malate + NAD^+
mitochondria:	malate + NAD^+ → OA + NADH + H^+
	OA + glutamate → aspartate + 2-OG

The driving force, which makes the MAS unidirectional toward NADH oxidation in the cytosol and its formation in mitochondria, is the unidirectional aspartate transfer from the mitochondria, due to the proton gradient created by the respiratory chain. Since the MDH is sensitive to the NADH to NAD^+ ratio, the flux through the MAS increases under conditions of

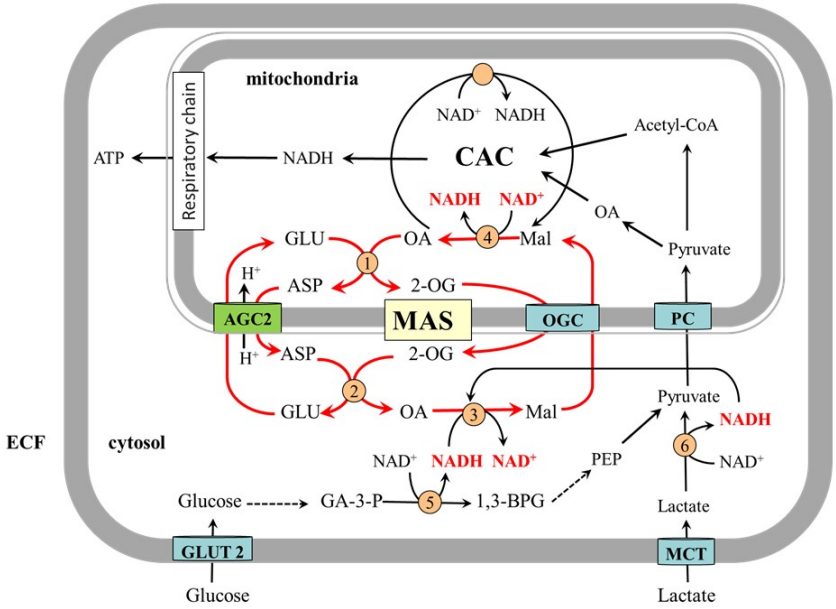

Fig. 2. MAS and its relationship to glycolysis and CAC in hepatocytes. 1, AST mitochondrial; 2, AST cytosolic; 3, malate dehydrogenase cytosolic; 4, malate dehydrogenase mitochondrial; 5, 3-phosphoglyceraldehyde dehydrogenase; 6, lactate dehydrogenase. AGC2, aspartate-glutamate carrier 2; ASP, aspartate; CAC, citric acid cycle; ECF, extracellular fluid; GA-3-P, glyceraldehyde-3-phosphate; GLU, glutamate; GLUT 2, glucose transporter 2; Mal, malate; MAS, malate-aspartate shuttle; MCT, monocarboxylate transporter; OA, oxaloacetate; OGC, oxoglutarate carrier; PC, pyruvate carrier; PEP, phosphoenolpyruvate; 1,3-BPG, 1,3-bisphosphoglycerate; 2-OG, 2-oxoglutarate.

increased NADH availability in the cytosol. This can occur when glycolysis is activated, such as after the increased intake of sugars in the food. If the supply of the cytosolic NADH decreases and/or mitochondrial NADH supply increases, for example due to the activation of beta-oxidation of fatty acids in mitochondria during the adapted phase of starvation and untreated type 1 diabetes, the flux through the MAS can decrease. Aspartate utilization will then be shifted in another direction, e.g., into the urea cycle or gluconeogenesis.

AGC2 IN THE UREA CYCLE AND GLUCONEOGENESIS

The scientific consensus is that most of the amino groups of the excess of amino acids, for example, during the first days of starvation, when a high-protein and low-carbohydrate diet is fed, and in disorders associated with muscle wasting, are converted into urea through the urea cycle, whereas their carbon skeletons are transformed mostly to glucose (15-17). Therefore, the pathways of urea synthesis and gluconeogenesis should be simultaneously regulated, and in balance. For proper understanding of the importance of AGC2 for simultaneous regulation of the urea cycle and gluconeogenesis, it is necessary to realize that:

(i) the two nitrogen atoms in the urea molecule come from two different sources, one being ammonia, the other being aspartate;
(ii) aspartate can act as a form for the indirect transport of OA, the initial substrate of gluconeogenesis, from mitochondria to the cytosol, where enzymes of gluconeogenesis are localized; and
(iii) the flux of aspartate and glutamate through AGC2, the urea cycle, and gluconeogenesis can be stimulated by Ca^{2+} due to the increased levels of hormones that acutely stimulate amino acid catabolism and gluconeogenesis, that is, glucagon, epinephrine, and norepinephrine (18).

Under conditions of enhanced amino acid catabolism, glutamate formation in the cytosol and aspartate synthesis from glutamate provided by AGC2 and OA formed from the carbon skeletons of gluconeogenic amino acids are stimulated. Hence, the flux through the urea cycle can increase due to the increased supply of both aspartate and ammonia that can be obtained from the blood, or synthesized in the mitochondria by glutaminase and glutamate dehydrogenase.

Increased flux through the urea cycle results in the enhanced synthesis of argininosuccinate that is cleaved by argininosuccinate lyase to arginine and fumarate. Arginine is converted by arginase to urea and ornithine, while fumarate leaves the urea cycle, and is subsequently converted by fumarate hydratase to malate. Malate can be converted to OA that can enter the gluconeogenesis pathway through phosphoenolpyruvate carboxykinase (PEPCK). When gluconeogenesis is stimulated,

the conversion of malate to OA can be facilitated by the increased supply of NAD^+ produced by 3-phosphoglyceraldehyde dehydrogenase (Fig. 3).

In summary, the simultaneous activation of the flux through the urea cycle and gluconeogenesis is ensured by AGC2 and the following sequence of enzymatic reactions:

1. Glutamate formation in the cytosol and its transport to mitochondria by AGC2.
   : amino acid + 2-OG → keto acid + glutamate
2. Asparate synthesis in mitochondria and its transport to the cytosol by AGC2.
   : glutamate + OA → 2-OG + aspartate
3. The entry of ASP into the urea cycle via argininosuccinate (AS) synthetase.
   : aspartate + citrulline + ATP → AS + ADP + Pi
4. Fumarate synthesis by AS lyase in the urea cycle.
   : AS → arginine + fumarate
5. Malate synthesis by fumarate hydratase.
   : fumarate + H_2O → malate
6. OA synthesis by malate dehydrogenase.
   : malate + NAD^+ → OA + NADH + H^+
7. OA entry into gluconeogenesis by PEPCK.
   : OA + GTP → PEP + GDP + CO_2

It should be emphasized that aspartate can also be directly converted to OA by the cytosolic AST (aspartate + 2-OG → OA + glutamate). However, under conditions where the main substrate for gluconeogenesis is amino acids, this pathway of OA synthesis seems unlikely, because it would interfere with aspartate entry into the urea cycle and ammonia detoxification. It can be assumed that OA synthesis from aspartate by the

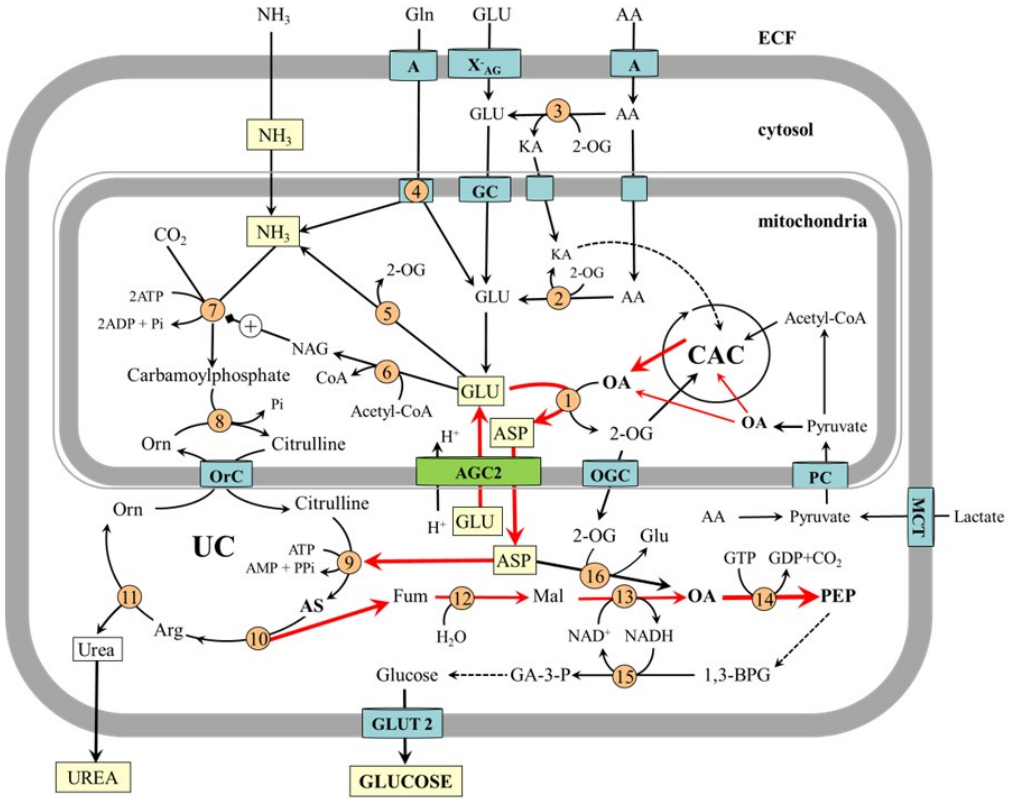

Fig. 3. Schematic role of AGC2 in synergistic regulation of urea cycle and gluconeogenesis. 1, AST mitochondrial; 2, aminotransferase (2-OG dependent) mitochondrial; 3, aminotransferase (2-OG dependent) cytosolic; 4, glutaminase; 5, glutamate dehydrogenase; 6, N-acetylglutamate synthase; 7, carbamoylphosphate synthetase; 8, ornithine carbamoyltransferase; 9, argininosuccinate synthetase; 10, argininosuccinate lyase; 11, arginase; 12, fumarate hydratase; 13, malate dehydrogenase cytosolic; 14, phosphoenolpyruvate carboxykinase; 15, 3-phosphoglyceraldehyde dehydrogenase; 16, AST cytosolic. A, amino acid transport system A; AA, amino acid; AGC2, aspartate-glutamate carrier 2; AS, argininosuccinate; ASP, aspartate; CAC, citric acid cycle; ECF, extracellular fluid; Fum, fumarate; GA-3-P, glyceraldehyde-3-phosphate; GC, glutamate carrier; GLU, glutamate; GLUT 2, glucose transporter 2; KA, keto acid; Mal, malate; MCT, monocarboxylate transporter; NAG, N-acetylglutamate; OA, oxaloacetate; OGC, oxoglutarate carrier; OrC, ornithine/citrulline carrier; PC, pyruvate carrier; PEP, phosphoenolpyruvate; Pi, inorganic phosphate; UC, urea cycle; X^-_{Ag}, amino acid transport system X-AG; 1,3-BPG, 1,3-bisphosphoglycerate; 2-OG, 2-oxoglutarate.

cytosolic AST and the subsequent use of OA for gluconeogenesis is preferred when the main substrate for gluconeogenesis is lactate. Even in this case, AGC2 should play a crucial role in the indirect delivery of OA to the cytosol for the needs of gluconeogenesis.

AGC2 AND PROTEIN SYNTHESIS

Since the main source of aspartate in the liver is its synthesis from OA in the mitochondria, the transport of aspartate from mitochondria to the cytosol by AGC2 is of fundamental importance for protein synthesis. Furthermore, in the reaction catalyzed by asparagine synthetase (aspartate + glutamine → asparagine + glutamate), aspartate is the exclusive substrate for the synthesis of asparagine, which is also proteinogenic amino acid. Both aspartate and asparagine are polar amino acids, the residues of which lie mainly on the surface of proteins, where they influence the folding of proteins and interactions among proteins, or play a role in the regulation of the activity of enzymes. The content of aspartate and asparagine in proteins is around 8 and 4%, respectively (19).

AGC2 AND CELL PROLIFERATION

Several studies have demonstrated that AGC expression and increased aspartate synthesis are specifically required in proliferating cells (10, 20, 21). This is because aspartate is an essential nitrogen donor for purine and pyrimidine synthesis, and thus for the synthesis of nucleotides, such as ATP, and nucleic acids (DNA and RNA). Furthermore, asparagine synthesized from aspartate by asparagine synthetase stimulates the activity and expression of ornithine decarboxylase, the rate-controlling enzyme in the biosynthesis of polyamines, e.g., spermine, spermidine, and putrescine, which are important players in the regulation of cell proliferation and differentiation (22).

Other studies have shown the importance of Ca^{2+} signals (23, 24) and Ca^{2+} mobilizing agents, such as noradrenaline and hepatocyte growth factor, for the progression of liver regeneration after partial hepatectomy (25, 26). Therefore, AGC2 and its stimulation by Ca^{2+} can ensure sufficient aspartate supply from mitochondria to the cytosol for the synthesis of nucleotides, nucleic acids, and proteins during liver regeneration in the case of liver damage.

AGC2 DEFICIENCY

AGC2 deficiency (citrullinemia type 2) is an autosomal recessive disease that can manifest in newborns as "neonatal intrahepatic cholestasis", in older children as "failure to thrive and dyslipidemia", and in adults as "recurrent hyperammonemia" (27, 28). However, some patients with AGC2 deficiency exhibit no obvious symptoms, and appear healthy (29). Several reports have described the onset of hepatocellular carcinoma in patients with AGC2 deficiency (30-32).

The major biochemical abnormalities in the liver due to AGC2 deficiency include increased NADH/NAD^+ ratio, decreased arginosuccinate synthetase activity, and low aspartate, malate, and OA levels in the cytosol. The alterations in plasma include citrullinemia, hypoproteinemia, hypoglycemia, galactosemia, hyperlipidemia, increased lactate/pyruvate ratio, and decreased concentrations of L-serine. Frequent manifestations include attacks of hypoglycemia and hyperammonemia, growth failure, hepatopathy, cholestasis, aversion to high-carbohydrate foods, and a preference for high-fat or high-protein food (29, 33, 34).

Several observations are explainable by the loss of AGC2 function and subsequent decrease in aspartate supply for the urea cycle, MAS, and the synthesis of proteins and nucleotides:

- The cause of recurrent hyperammonemia should be impaired ammonia detoxification to urea due to decreased aspartate supply to the urea cycle. The consequences related to impaired flux through the urea cycle are citrullinemia (due to lack of aspartate for argininosuccinate synthesis), and decreased gluconeogenesis via fumarate.
- Observations of hyperammonemia after excess of carbohydrates in the diet (28) can be explained by the rise in the level of NADH formed in the cytosol during glycolysis, and subsequent shift from the use of a limited amount of aspartate from its use in the urea cycle to the MAS. The suggestion is supported by the beneficial influence of medium-chain triglyceride supplementation leading to a reduction in the cytosolic NADH/NAD^+ ratio (35).
- The dislike of carbohydrates and sweets of patients with AGC2 deficiency may be related to impaired flux through MAS.
- It has been suggested that a role in hepatopathy and hyperlipidemia plays impaired flux through MAS that is compensated by up-regulation of the malate-citrate shuttle that increases citrate supply to the cytosol and the subsequent overproduction of fatty acids (36).
- Hypoglycemic attacks can be related to the decrease in dietary carbohydrates and impaired gluconeogenesis.
- Besides the impaired supply of aspartate for OA synthesis, a role in decreased gluconeogenesis can result in impaired conversion of lactate to pyruvate due to the increased NADH/NAD^+ ratio in the cytosol.
- The likely cause of the decrease in L-serine concentration is the decreased synthesis of 3-phosphoglycerate, which is an intermediate in the glycolysis and gluconeogenesis pathways and the main endogenous precursor of L-serine (37).

AGC2 AND CANCER

Gene expression data from The Cancer Genome Atlas have shown that cancer cells adapt metabolism to their microenvironment, and metabolize amino acids more economically by optimizing the gene expression profile (38). Obviously, these tumor-promoting mutations provide a growth advantage to

highly proliferating cells over healthy cells.

Several reports have indicated that AGC responds to the influence of oncogenes, and its expression is markedly enhanced in tumors (39-42). In this context, aspartate has been described as a limiting metabolite for cancer cell growth (21, 43); Amodeo *et al*. have underlined the role of AGC expression in maintaining the cell redox balance, mitochondrial respiration, and ATP synthesis via MAS and glutathione regeneration by tumor cells (40).

Overexpression of AGC2 was found in human colorectal cancer and patients with melanoma (39, 42). Other studies have shown that AGC2 deficiency (citrullinemia type 2) significantly increases the risk of liver cancer (30-32, 44, 45). In this context, it has been reported that SLC25A12 gene (AGC1 expression) is reactivated in hepatocellular carcinoma through histone acetylation and cAMP response-element binding protein (CREB) recruitment, and that SLC25A12 silencing by small interfering RNA significantly impaired HepG2 cell proliferation (46). Hence, it has been suggested that the upregulation of AGC1 (CLC25A12, aralar 1) plays a role in carcinogenesis in patients with AGC2 (SLC25A13) deficiency (46, 47). All these studies indicate AGC as a potential therapeutic target to fight cancer (48, 49).

SUMMARY AND CONCLUSIONS

The role of AGC2 in the compartmentalization of aspartate and glutamate metabolism between mitochondria and the cytosol makes AGC2 a unique player in intermediary metabolism. AGC2 via MAS is essential for the aerobic oxidation of glucose and lactate conversion to pyruvate, intracellular redox balance, mitochondrial respiration, and ATP synthesis. The supply of glutamate produced during amino acid catabolism in the cytosol to mitochondria is essential for the synthesis of ammonia by glutamate dehydrogenase and aspartate synthesis from OA and glutamate by mitochondrial AST. Aspartate transport from mitochondria into the cytosol is of crucial importance for the urea cycle, synthesis of nucleotides and proteins, gluconeogenesis, and cell proliferation.

Through elevated Ca^{2+} levels, the AGC2 is probably stimulated during fasting, exhaustive exercise, and muscle wasting disorders. The result of increased aspartate input to the cytosol is stimulation of the urea cycle and fumarate synthesis, which can be converted to malate, and then to OA, a common substrate for gluconeogenesis. The conversion of malate to OA can be ensured by the decrease in the NADH/NAD^+ ratio due to the activation of gluconeogenesis by glucagon or catecholamines.

In conclusion, AGC2 plays a crucial role in the compartmentalization of aspartate and glutamate metabolism between mitochondria and the cytosol, and linking the reactions of amino acid catabolism, urea cycle, gluconeogenesis, protein synthesis, and cell proliferation. The targeting of AGC genes to limit aspartate supply from mitochondria to the cytosol may represent a new strategy to fight cancer.

ACKNOWLEDGEMENTS

This work was supported by Charles University, the Cooperatio Program, research area METD.

CONFLICTS OF INTEREST

The author have no conflicting interests.

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