Citrin deficiency: Does the reactivation of liver aralar-1 come into play and promote HCC development?

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HYPOTHESIS

Citrin deficiency: Does the reactivation of liver aralar-1 come into play and promote HCC development?

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ABSTRACT

Hepatocellular carcinoma (HCC) is a longstanding issue in clinical practice and metabolic research. New clues in better understanding the pathogenesis of HCC might relate to the metabolic context in patients with citrin (aspartate-glutamate carrier 1) deficiency (CD). Because citrin-deficient liver (CDL) is subject to HCC, it represents a unique metabolic model to highlight the mechanisms of HCC promotion, offering different angles of study than the classical metabolic syndrome/obesity/non-alcoholic fatty liver disease (NAFLD)/HCC study axis. In turn, the metabolic features of HCC could shed light on the pathogenesis of CDL. Among these, HCC-induced re-activation of aralar-1 (aspartate-glutamate carrier 2), physiologically not expressed in the adult liver, might take place in CDL, so gene redundancy for mitochondrial aspartate-glutamate carriers would be exploited by the CDL. This proposed (aralar-1 re-activation) and known (citrate/malate cycle) adaptive mechanisms may substitute for the impaired function in CD and are consistent with the clinical remission stage of CD and CD improvement by medium-chain triglycerides (MCT). However, these metabolic adaptive benefits could also promote HCC development. In CD, as a result of PPARα down-regulation, liver mitochondrial fatty acid-derived acetyl-CoA would, like glucose-derived acetyl-CoA, be used for lipid anabolism and fuel nuclear acetylation events which might trigger aralar-1 re-activation as seen in non-CD HCC. A brief account of these metabolic events which might lead to aralar-1 re-activation in CDL is here given. Consistency of this account for CDL events further relies on the protective roles of PPARα and inhibition of mitochondrial and plasma membrane citrate transporters in non-CD HCC.

Keywords: mitochondrial solute carriers; SLC25A1; SLC25A12; SLC25A13; citrin; aralar-1; malate-aspartate shuttle; citrate-malate shuttle; cytosolic NADH redox state; histone acetylation; gene redundancy; gene re-activation; citrin deficiency; hepatocellular carcinoma

1. Introduction

Hepatocellular carcinoma (HCC) is a long-standing problem in clinical practice and research. It is the leading type of liver cancer, accounting for approximately 4 out of 5 liver cancers and is the second leading cause of death in cancer patients [1]. Current concepts and future challenges in HCC have been reviewed notably by considering the metabolic syndrome/obesity/non-alcoholic fatty liver disease (NAFLD)/HCC study axis, with a special emphasis on the burden of NAFLD-associated HCC [1]. Citrin deficiency (CD) is rare inborn error of metabolism and patients with CD may develop HCC [2] sometimes in the infant age [3]. Because citrin-deficient liver (CDL) is subject to HCC, it represents a unique metabolic model to highlight the mechanisms of HCC promotion, offering different angles of study than the metabolic syndrome/obesity/NAFLD/HCC study axis mentioned above. In turn, the metabolic features of HCC could shed light on the pathogenesis of CDL. Among these, the re-activation of aralar-1 seen in non-CD HCC [4] might be speculated to take place in CDL. Though in CDL, aralar-1 re-activation might have obvious metabolic benefits, it might also contribute to HCC development in CD. A brief discussion of the metabolic background that supports this proposal is presented here.

2. The two aspartate/glutamate carriers and citrin deficiency

Aspartate/glutamate exchange is catalyzed by proteins located in the mitochondrial inner membrane and activated by calcium acting at the external face of this membrane [5]. Depending on the cell type, this exchange may be catalysed by citrin (SLC25A13), aralar-1 (SLC25A12), or both proteins [6]. The metabolic basis and treatment of patients exhibiting CD was recently reviewed as well as the different phenotypes including severe neonatal and adult onset presentations, and a third compensation and adaption (remission-like) phenotype developing between neonatal and adult ages [2]. As mentioned above, CD is also a cause of HCC [2].

3. Rise of cytosolic NADH in CDL may be partially compensated by the citrate/malate cycle

In CDL, high cytosolic NADH/NAD^+ ratio, resulting from the failure to shuttle NADH (*via* malate) between cytosol and mitochondria, is a key driver of the citrate/malate cycle [7]. This cycle starts with the mitochondrial export of citrate to the cytosol where it is cleaved by ATP-citrate lyase (ACLY) to oxaloacetate and acetyl-CoA. As a result of high cytosolic NADH levels, oxaloacetate is reduced to malate which fuels back mitochondria Krebs'cycle to regenerate citrate. The citrate/malate cycle can act as an energy dependent pathway which drives the oxidation of cytosolic NADH [8], and hence might partially substitute for citrin. The roles of citrin and citrate/malate cycle in cytosol/mitochondria NADH redox shuttling are illustrated in Figure 1.

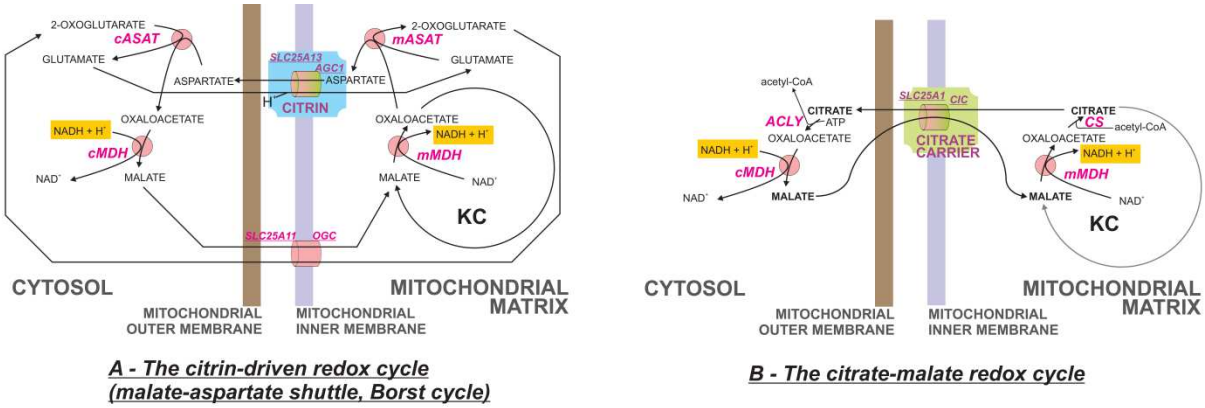

Figure 1 – Mitochondrial/cytosolic redox shuttling of NADH with an emphasis on the citrin (A) and citrate/malate (B) redox shuttles. Note the citrin-driven redox cycle is also referred to as the malate/aspartate shuttle and, historically, initially to as the Borst cycle. The citrate/malate (B) *vs* citrin-catalyzed (A) cycles differ by the mitochondrial export of acetyl-CoA and need of ATP for the citrate/malate shuttle (illustrated), and in liver, by the involvement of citrin in the urea cycle (not illustrated). Abbreviations are: KC, Krebs cycle; mMDH, mitochondrial malate dehydrogenase; cMDH, cytosolic malate dehydrogenase; mASAT, mitochondrial aspartate aminotransferase; cASAT, cytosolic aspartate aminotransferase; CS, citrate synthase; ACLY, ATP citrate lyase. A pragmatic and historical account for the two illustrated NADH redox shuttles may be found in the recent review of Piet Borst cited in the main text.

4. MCT and CDL mitochondrial acetyl-CoA

The reason why MCT supplementation is implemented in CD patients is to provide patients with a diet energy supply which lowers or removes sugar uptakes. This avoidance of glucose is justified by the known promoting effect of glucose on liver cytosolic NADH burst and hence toxic expression of the disease [2, 7]. Therein, patients with CD are currently proposed to be all candidates for being given MCT [2]. Under MCT supplementation, CDL is supplied with MCFA directly *via* the portal circulation [2]. MCFA bypass the carnitine palmitoyltransferase type 1 (CPT1) step inhibited by malonyl-CoA (secondary to high cytosolic acetyl-CoA levels resulting from the stimulation of citrate/malate cycle), and afford mitochondrial energy yield and formation of acetyl-CoA. In the CDL, PPARα mRNA levels are decreased as well as the protein levels (measured by immunoblot detection) and function (assayed by DNA-binding activity) [9]. These changes are majored under steatosis and proposed to result possibly from stimulations of c-Jun-N-terminal kinase (JNK) and inflammation signalling [9]. Clinically, circulating markers consistent with PPARα deficiency (or at least biological changes targeted by PPARα agonists) are sometimes, but not always, described in patients with CD. These may include hypertriglyceridemia [10] and its lowering by the pan-PPAR agonist bezafibrate [11], and in CD patients with echinocytosis, low levels of HDL-cholesterol and ApoA1 [12]. Clinical development of hypoketotic hypoglycaemia [13] as seen in mitochondrial β-oxidation disorders [14]

might be accounted for by impairment of this pathway secondarily to PPARα deficiency. Biochemically, indeed, the PPARα deficiency is, in CDL, associated with down-regulation of the expression of PPARα target genes and resulting decrease in the levels of proteins involved in mitochondrial and peroxisomal fatty acid oxidation [9]. This may reduce the fuelling of ketone body synthesis. In parallel, ketogenesis enzymes might be directly impacted. In CDL, the down-regulation of PPARα should affect ketogenesis by the loss of the up-regulation by this nuclear receptor of the mitochondrial 3-hydroxy,3-methylglutratyl-CoA (HMG-CoA) synthase (HMGS2), a key enzyme in ketone body synthesis [15]. Accordingly in citrin-deficiency, circulating ketone bodies acetoacetate and β-hydroxybutyrate are low [9,13] and are expected to be increased by MCT to a lower extent than normal. Since less committed in ketogenesis, fatty acid-derived acetyl-CoA might fuel the citrate/malate cycle. Along with the drop in dietary intake of glucose, this might contribute to the improvement by MCFA of cytosolic NADH levels in CDL [2].

5. MCT and CDL nuclear acetyl-CoA

Improvement of cytosolic NADH levels reported for MCT was also proposed for pyruvate in CD [2]. Interestingly, MCT and pyruvate could fuel nuclear acetyl-CoA. In CDL, MCT might increase nuclear acetylating events *via* stimulation of the malate/citrate cycle. Indeed, ACLY is a cytosolic-nuclear enzyme which may directly cleave citrate in the nucleus to provide acetyl-CoA for nuclear acetylating reactions [16,17]. Pyruvate can directly fuel acetyl-CoA for histone acetylation *via* nuclear pyruvate dehydrogenase [16].

6. Aralar-1 reactivation: a additional metabolic adaptive mechanism in CDL?

Aralar-1, the other mitochondrial aspartate-glutamate carrier, is physiologically expressed in many adult cell types but not hepatocytes [6]. Interestingly, liver aralar-1 gene expression may be reactivated. This has been observed in HCC and promoted by increased histone acetylation and cAMP Response Element-Binding Protein (CREB) recruitment [4]. Therapeutic mechanisms conveyed by MCT and pyruvate could rely on increased histone acetylation which might trigger liver *SLC25A12* reactivation.

7. Aralar-1 reactivation as a HCC promoting event in CDL?

Aralar-1 reactivation might result from the increased fueling of nuclear acetyl-CoA in CDL. The increased acetylated histones may also down-regulate arginosuccinate synthetase 1 (ASS1) gene [18,19]. This down-regulation might little or no affect ammonia removal which in CDL rely more on glutamine synthetase than on urea cycle [2]. By preventing the use of aspartate for ammonia

detoxifying purposes, it might however drive aspartate use towards protein synthesis, and nucleotide biosynthesis. Overall, this might contribute to promoting tumoral development, even if in CD, impaired ASS1 is subject to heterogeneity, respective to liver regions [20]. As stressed above, aralar-1 reactivation has been described in non-CD HCC (HepG2 cells) [4], CD is a cause of HCC [2,3] and mitochondrial dysfunction (loss of both citrin function itself and PPARα function) [9], and aralar-1 is only found in the fetal/developing liver [6]. Interestingly, the stem cell phenotype of HCC HepG2 cells has recently been shown to be induced (increased stem markers) by nandrolone via mitochondrial dysfunction (inhibition of mitochondrial OXPHOS complexes) [21]. Therefore, although not pointed in the latter study, it is not unreasonable to think that reactivation of aralar-1 could be a pioneer stem marker or, as suggested for α-fetoprotein [22], could promote HCC development and metastasis and possibly HCC stem cell initiation. As suggested for norandrolone-induced mitochondrial dysfunction [21], mitochondrial impairment in the CDL (combining deficiency of citrin function and PPARα-dependent pathways) could also represent a precondition that, in addition to multiple impairments, could expose to HCC development. Further studies focusing on aralar-1 in liver stem cells should clarify this issue. In addition to an action on HCC initiation, aralar-1 provides cells with a metabolic reprogramming that, in combination with the reduced CDL content in ASS-1 (see above), could increase the supply of nucleic acid and protein syntheses with aspartate. Thus, aralar-1 could not only pre-expose to HCC development but also promote it.

8. Conclusion and perspective for future study

In CDL, links between metabolic reprogramming and nuclear acetylation events still need clarifications, notably whether the reactivation of liver aralar-1 might contribute to improvement by therapies, and remission stage of the disease. Under this consideration, though antitumoral role of MCT was supported by suggested down-regulated Wnt/β-catenin axis and a favourable issue in one case in CD [20], re-expression of aralar-1 in CD (and possibly potentiated by MCT) might unprotect since in HCC this re-activation drives the use of aspartate towards protein and nucleotide biosynthesis thereby promoting tumoral development [4]. By substituting for citrin, the re-activation of aralar-1 might explain the remission (adaptation and compensation) stage observed in this disease, and possibly the metabolic benefit of MCT. Any MCT activity on HCC development in CD might be explored by monitoring more systematically circulating alpha-fetoprotein (as a biological, though not specific, marker of hepatocellular carcinoma) in MCT-treated and untreated patients with CD. Despite the limited number of patients with CD, measurements of circulating α-fetoprotein are feasible and promoted by widespread implementation in clinical chemistry laboratories in the medical routine practice. Such measurements are therefore readily available in the screening and follow-up setting to monitor changes induced by therapeutic measures and disease course. Alpha-

fetoprotein measurements can even be performed on dried blood spots, improving compliance of patients and reducing costs for the health care screening [23].

Present considerations on CD are well consistent with the protective roles of PPARα [24] and combined inhibition of mitochondrial and plasma membrane citrate transporters [25] in HCC. Finally, studies on viable models of citrin-deficient cells, even if they are not hepatocytes (little or no ketogenesis would approximate CDL instead of moving away from it), such as those obtained with CRISPR/Cas9 technology to induce SLC25A13 knockout [26] should also be useful to study the effect of MCT (or MCFA) on nuclear acetylation events, and therein contribution of other (acetylcarnitine and acetate instead of citrate) routes linking mitochondrial and nuclear acetyl-CoA metabolism. To our knowledge, in contrast to non-CD HCC [4], there are still no currently data directly supporting the re-activation of aralar-1 in response to citrin gene inactivation or in CD HCC. Knockout models of human CD are expected to prone to develop HCC and might help exploration of the possible promoting effects of MCT. In this respect, mice with *SLC25A13 (citrin)*-knockout gene only partially but not fully mimics the pathogenesis of human CD [27]. By contrast, the citrin/mitochondrial glycerol-3-phosphate dehydrogenase (mG3PDH) double-knockout mouse model rather accounts properly for the human CD [28]. This latter model should be thus more suitable to study aralar-1 reactivation and HCC development. A current account for this matter in the double knockout mouse model is, however, still awaited. The reason why a murine double knock-out is needed to mimic the human CD might rely on a prominant role of citrin over mG3PDH to control cell NADH redox states in the human liver whereas in mouse liver this control might be shared in similar or complementary parts by the two mitochondrial (citrin and G3PDH) proteins.

The main hypothesis of this article is that aralar-1 might be re-activated in the CDL. In the proposed metabolic context on which aralar-1 re-activation could rely, how MCT/MCFA might be exactly metabolized and, hence, how they might impact HCC development are currently unknown. Thus, as regards to MCT therapy in human CD, the possible HCC promoting risk should deserve a very special attention in view of the combination of mitochondrial metabolic and cytosolic redox disturbances which, in CDL, might orientate MCT metabolism towards a fuelling of nuclear hyperacetylating events. As mentioned above, these events are known to promote re-activation of aralar-1, HCC development, and hence increased liver secretion of α-fetoprotein (notably increased HCC-associated forms of this protein including the L3 fraction [29] and the aberrant osylated forms [30, 31]). Though MCT therapy is *a priori* an adapted and convenient therapy, the risk for its pro-HCC promoting effects in patients with CD should be urgently checked forwards (*via* monitoring of the HCC α-fetoprotein markers) in the special case of patients with CD to reposition, if needed, the recommendation of this therapeutic measure in this disease. Other therapies based on glucose (ethanol and glycerol) avoidance or slowly carbon hydrate releasing forms (to avoid exposure of liver to a burst of glucose), and administration of a PPARα agonist drug such as fenofibrate (to restore the lost connexion between urea cycle function, PPARα signalling, and fatty acid oxidation mentioned in

[9]) remain therapeutic options that warrants future consideration in treating patients with CD. Ketone body administration and, in view of the long known liver peroxisome role in electron shuttling and content in lactate oxidase [32], L-lactate-based therapy should not be disregarded.

Author Contributions

Each author contributes to the rationale of the manuscript during clinico-biological weekly seminars on the follow-up of patients with inherited metabolic disorders at the University Hospital of Lille, with drafting of the manuscript by Joseph Vamecq. All authors approved the final version of the manuscript.

Acknowledgments

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REFERENCES

[1] Q.M. Anstee, H.L. Reeves, E. Kotsiliti, O. Govaere, M. Heikenwalder, From NASH to HCC: current concepts and future challenges. Nat Rev Gastroenterol Hepatol 16 (2019) 411-428.

[2] K. Hayasaka, Metabolic basis and treatment of citrin deficiency. J Inherit Metab Dis 44 (2021) 110-117.

[3] J. He, J. Zhang, X. Li, H. Wang, C. Feng, F. Fang, S. Shu, A Case Report: Can Citrin Deficiency Lead to Hepatocellular Carcinoma in Children? Front Pediatr 7 (2019) 371.

[4] V. Infantino, F. Dituri, P. Convertini, A. Santarsiero, F. Palmieri, S. Todisco, S. Mancarella, G. Giannelli, V. Iacobazzi, Epigenetic upregulation and functional role of the mitochondrial aspartate/glutamate carrier isoform 1 in hepatocellular carcinoma. Biochim Biophys Acta Mol Basis Dis 1865 (2019) 38-47.

[5] L. Palmieri, B. Pardo, F.M. Lasorsa, A. del Arco, K. Kobayashi, M. Iijima, M.J. Runswick, J.E. Walker, T. Saheki, J. Satrústegui, F. Palmieri, Citrin and aralar1 are Ca(2+)-stimulated aspartate/glutamate transporters in mitochondria. EMBO J 20 (2001) 5060-5069.

[6] A. del Arco, J. Morcillo, J.R. Martínez-Morales, C. Galián, V. Martos, P. Bovolenta, J. Satrústegui, Expression of the aspartate/glutamate mitochondrial carriers aralar1 and citrin during development and in adult rat tissues. Eur J Biochem 269 (2002) 3313-3320.

[7] N.C. Lee, Y.H. Chien, K. Kobayashi, T. Saheki, H.L. Chen, P.C. Chiu, Y.H. Ni, M.H. Chang, W.L. Hwu, Time course of acylcarnitine elevation in neonatal intrahepatic cholestasis caused by citrin deficiency. J Inherit Metab Dis 29 (2006) 551-555.

[8] P. Borst, The malate-aspartate shuttle (Borst cycle): How it started and developed into a major metabolic pathway. IUBMB Life 72 (2020) 2241-2259.

[9] M. Komatsu, T. Kimura, M. Yazaki, N. Tanaka, Y. Yang, T. Nakajima, A. Horiuchi, Z.Z. Fang, S. Joshita, A. Matsumoto, et al., Steatogenesis in adult-onset type II citrullinemia is associated with down-regulation of PPARα. Biochim Biophys Acta 1852 (2015) 473-481.

[10] M. Komatsu, M. Yazaki, N. Tanaka, K. Sano, E. Hashimoto, Y. Takei, Y.Z. Song, E. Tanaka, K. Kiyosawa, T. Saheki, T. Aoyama, K. Kobayashi, Citrin deficiency as a cause of chronic liver disorder mimicking non-alcoholic fatty liver disease, J. Hepatol. 49 (2008) 810–820.

[11] R. Terada, K. Yamamoto, K. Kobayashi, K. Sakaguchi, Y. Iwasaki, T. Saheki, Y. Shiratori, Adult-onset type II citrullinemia associated with idopathic hypertriglyceridemia as a preceding feature, J. Gastroenterol. Hepatol. 21 (2006) 1634–1635.

[12] Y.Z. Song, M. Deng, F.P. Chen, F. Wen, L. Guo, S.L. Cao, J. Gong, H. Xu, G.Y. Jiang, L. Zhong, K. Kobayashi, T. Saheki, Z.N., Wang Genotypic and phenotypic features of citrin deficiency: five-year experience in a Chinese pediatric center. Int J Mol Med 28 (2011) 33-40.

[13] Y. Wada, N. Arai-Ichinoi, A. Kikuchi, O. Sakamoto, S. Kure, Hypoketotic hypoglycemia in citrin deficiency: a case report. BMC Pediatr 20 (2020) 444.

[14] R.J.A. Wanders, G. Visser, S. Ferdinandusse, F.M. Vaz, R.H. Houtkooper, Mitochondrial Fatty Acid Oxidation Disorders: Laboratory Diagnosis, Pathogenesis, and the Complicated Route to Treatment. J Lipid Atheroscler 9 (2020) 313-333.

[15] J.C. Rodríguez, G. Gil-Gómez, F.G. Hegardt, D. Haro, Peroxisome proliferator-activated receptor mediates induction of the mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene by fatty acids. J Biol Chem 269 (1994) 18767-18772.

[16] V.C. de Boer, S.M. Houten, A mitochondrial expatriate: nuclear pyruvate dehydrogenase. Cell 158 (2014) 9-10.

[17] K.E. Wellen, G. Hatzivassiliou, U.M. Sachdeva, T.V. Bui, J.R. Cross, C.B. Thompson, ATP-citrate lyase links cellular metabolism to histone acetylation. Science 324 (2009) 1076-1080.

[18] R. Lin, Y. Mo, H. Zha, Z. Qu, P. Xie, Z.J. Zhu, Y. Xu, Y. Xiong, K.L. Guan, CLOCK Acetylates ASS1 to Drive Circadian Rhythm of Ureagenesis. Mol Cell 68 (2017) 198-209.e6.

[19] W.B. Tsai, Y. Long, J.T. Chang, N. Savaraj, L.G. Feun, M. Jung, H.H.W. Chen, M.T. Kuo, Chromatin remodeling system p300-HDAC2-Sin3A is involved in Arginine Starvation-Induced HIF-1α Degradation at the ASS1 promoter for ASS1 Derepression. Sci Rep 7 (2017) 10814.

[20] K. Hayasaka, C. Numakura, M. Yamakawa, T. Mitsui, H. Watanabe, H. Haga, M. Yazaki, H. Ohira, Y. Ochiai, T. Tahara, et al., Medium-chain triglycerides supplement therapy with a low-carbohydrate formula can supply energy and enhance ammonia detoxification in the hepatocytes of patients with adult-onset type II citrullinemia. J. Inherit. Metab Dis 41 (2018) 777-784.

[21] F. Agriesti, T. Tataranni, C. Pacelli, R. Scrima, I. Laurenzana, V. Ruggieri, O. Cela, C. Mazzoccoli, M. Salerno, F. Sessa, G. Sani, C. Pomara, N. Capitanio, C. Piccoli, Nandrolone induces a stem cell-like phenotype in human hepatocarcinoma-derived cell line inhibiting mitochondrial respiratory activity. Sci Rep 10 (2020) 2287.

[22] Y. Zheng, M. Zhu, M. Li, Effects of alpha-fetoprotein on the occurrence and progression of hepatocellular carcinoma. J Cancer Res Clin Oncol 146 (2020) 2439-2446.

[23] A. Mussa, V.P. Ciuffreda, P. Sauro, V. Pagliardini, S. Pagliardini, D. Carli, J.M. Kalish, F. Fagioli, E. Pavanello, G.B. Ferrero, Longitudinal Monitoring of Alpha-Fetoprotein by Dried Blood Spot for Hepatoblastoma Screening in Beckwith¯Wiedemann Syndrome. Cancers (Basel) 11 (2019) 86.

[24] Q. Zhang, Y. Zhang, S. Sun, K. Wang, J. Qian, Z. Cui, T. Tao, J. Zhou, ACOX2 is a prognostic marker and impedes the progression of hepatocellular carcinoma via PPARα pathway. Cell Death Dis 12 (2021) 15.

[25] W.A. Poolsri, P. Phokrai, S. Suwankulanan, N. Phakdeeto, P. Phunsomboon, D. Pekthong, L. Richert, S. Pongcharoen, P. Srisawang, Combination of Mitochondrial and Plasma Membrane Citrate Transporter Inhibitors Inhibits De Novo Lipogenesis Pathway and Triggers Apoptosis in Hepatocellular Carcinoma Cells Biomed Res Int (2018) 2018:3683026.

[26] A.A. Shuvarikov, M.A. Davis, K. Esser-Nobis, M.J. Gale, Mitochondrial SLC25 proteins interact with NLRP3 to regulate inflammasome function. J Immunol 200 (1 Supplement) (2018) 115.8.

[27] D.S. Sinasac, M. Moriyama, M.A. Jalil, L. Begum, M.X. Li, M. Iijima, M. Horiuchi, B.H. Robinson, K. Kobayashi, T. Saheki, L.C. Tsui, Slc25a13-knockout mice harbor metabolic deficits but fail to display hallmarks of adult-onset type II citrullinemia. Mol Cell Biol 24 (2004) 527-536.

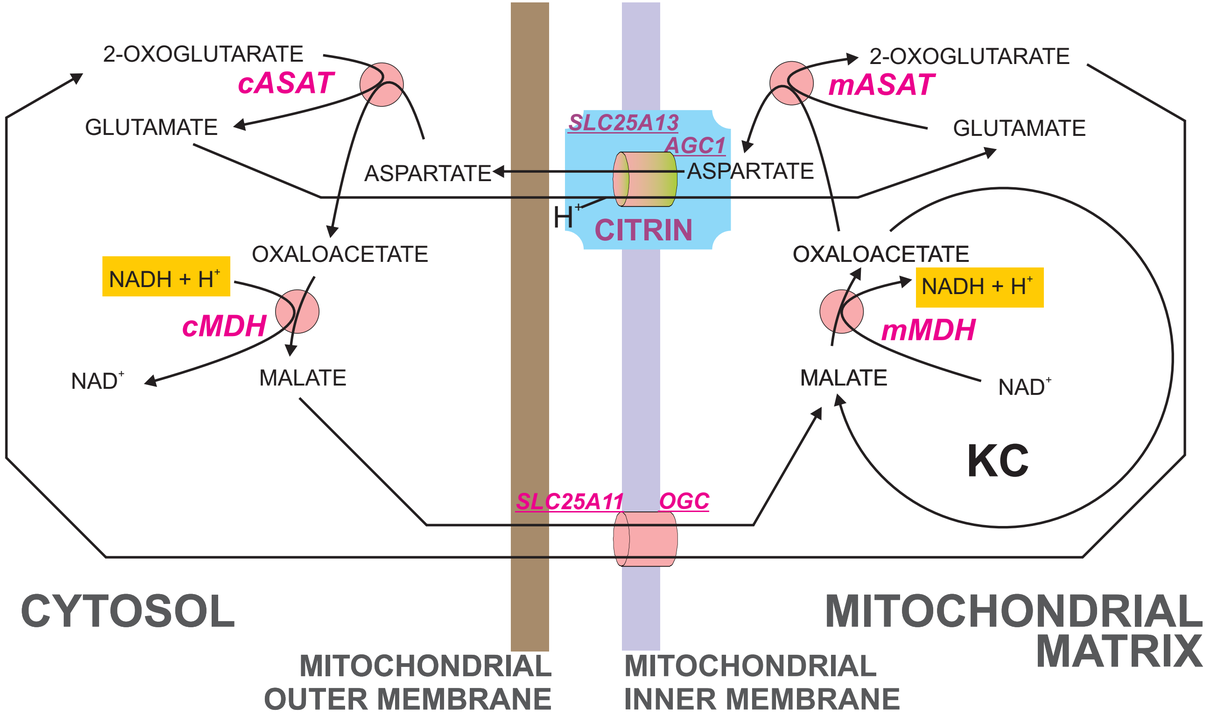
[28] T. Saheki, K. Inoue, H. Ono, N. Katsura, M. Yokogawa, Y. Yoshidumi, S. Furuie, E. Kuroda, M. Ushikai, A. Asakawa, A. Inui, K. Eto, T. Kadowaki, D.S. Sinasac, K. Yamamura, K. Kobayashi, Effects of supplementation on food intake, body weight and hepatic metabolites in the citrin/mitochondrial glycerol-3-phosphate dehydrogenase double-knockout mouse model of human citrin deficiency. Mol Genet Metab 107 (2012) 322-329.

[29] H.M. Ibrahim, M.Z.Elghannam, O.A.Y. Elkhawaga, A.M.A. El-Sokkary, Evaluation of serum alpha fetoprotein-L3 as an accuracy novel biomarker for the early diagnosis of hepatocellular carcinoma in Egyptian Patients. Saudi J Biol Sci (2021), in press

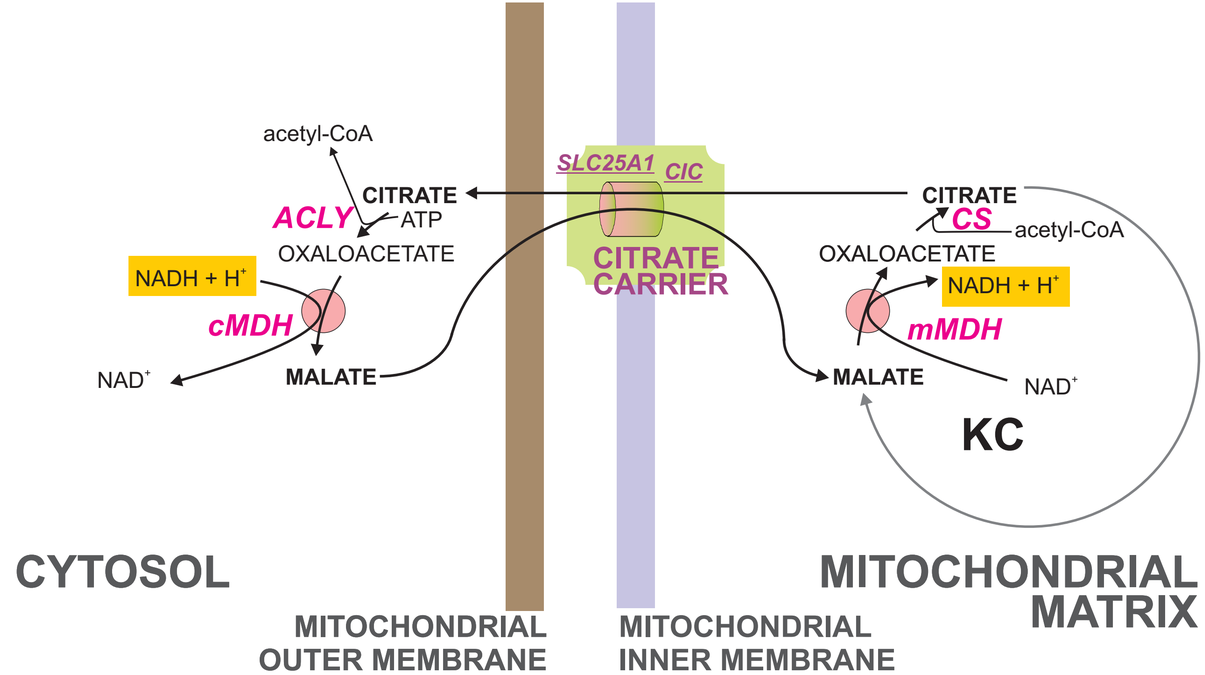
[30] K.H. Kim, S.Y. Lee, D.G. Kim, S.Y. Lee, J.Y. Kim, J.S.Yoo, Absolute Quantification of N-Glycosylation of Alpha-Fetoprotein Using Parallel Reaction Monitoring with Stable Isotope-Labeled N-Glycopeptide as an Internal Standard. Anal Chem 92 (2020) 12588-12595.

[31] K.H. Kim, S.Y. Lee, H. Hwang, J.Y. Lee, E.S. Ji, H.J. An, J.Y. Kim, J.S.Yoo, Direct Monitoring of Fucosylated Glycopeptides of Alpha-Fetoprotein in Human Serum for Early Hepatocellular Carcinoma by Liquid Chromatography-Tandem Mass Spectrometry with Immunoprecipitation. Proteomics Clin Appl 12 (2018) e1800062.

[32] C. De Duve, P. Baudhuin , Peroxisomes (microbodies and related particles). Physiol Rev 46 (1966) 323-357.



A - The citrin-driven redox cycle (malate-aspartate shuttle, Borst cycle)



B - The citrate-malate redox cycle