



Branched Chain Amino Acid Ketones, or Ketolysis of Beta Hydroxybutyrate Support Lipogenesis and Tumour Growth

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ABSTRACT

The glycolytic and fatty acid sources of mitochondrial acetyl-CoA being impaired or inhibited in tumour cells, they become dependent of ketone bodies for its production and for sustaining their metabolism. The ketone bodies are provided by liver ketogenesis or from ketogenic branched-chain amino acids (BCAA) to produce acetyl-CoA in tumour cells. Two pathways are involved: the ketolytic SCOT-oxct1 route, and the BCAA transaminase - keto dehydrogenase pathway. Acetyl-CoA and in fine succinyl CoA are formed. The two pathways converge at the lipogenic route, supporting tumour progression.

Keywords: SCOT-oxct1, BCAA, ketone-dependent tumours, lipogenic pathway

INTRODUCTION

We have previously pointed that an increased influx of glucose in tumour cells, with lactic acid fermentation and release, in presence of oxygen (Warburg effect) [1] could be a consequence of an inhibition of the last enzyme of glycolysis, the Pyruvate kinase step, in which the PK M2 [2] embryonic isoform of the enzyme is expressed and blocked by phosphorylation, failing to form active tetramers. The increased glucose uptake and glycolysis in tumour cells aims to overcome this PK (M2) bottle neck. In addition, the Pyruvate dehydrogenase (PDH) entry in mitochondrial oxidative metabolism, seems doubly locked by phosphorylation [3] and acetylation [4], forming a second bottle neck at the entry in oxidative metabolism. Hence, the pyruvate accumulated above PDH; is converted by lactate dehydrogenase into lactic acid and released. The glycolytic supply of acetyl-CoA to the citric acid cycle in mitochondria is greatly affected by this anomaly failing to unlock PK M2 and PDH. On the other hand, we know that the citric acid cycle in mitochondria normally forms citrate, the condensation of acetyl-CoA and oxaloacetate into citrate takes place, and citrate exits the mitochondria, feeding the fatty acid synthesis pathway and lipogenesis, forming new lipid membranes for mitotic tumour cells. Since the fatty acid synthesis route, via ATP citrate lyase (ACLY) and acetyl-CoA carboxylase (ACC) produces malonyl CoA (the product of ACC), the latter will automatically turn-off the fatty acid degradation, if fatty acid synthesis takes place, because malonyl CoA inhibits the fatty acid carnityl transporter and their entry in mitochondria, where their degradation by beta oxidation into acetyl-CoA takes place. Hence, in a mitotic cell synthesizing

its membranes, the fatty acid source of acetyl-CoA is closed [5]. The other possible source of acetyl-CoA in tumour cells are ketone bodies produced in the liver. Beta hydroxybutyrate, (BHB) is incorporated by tumour cells, converted into acetoacetate, then a specific ketolytic enzyme: Succinyl-CoA: oxoacid, CoA transferase (SCOT) forms acetoacetylCoA. An acetyl-CoA acetyltransferase (ACAT1) will produce two molecules of acetyl-CoA in the presence of CoA. In this way, the citric acid cycle condensation reaction forms citrate in tumour cells [6, 7]. Another source of acetyl-CoA comes from ketogenic branched chain amino acids (BCAA), (leucine isoleucine and valine). We previously showed that ketolysis and SCOT inhibition, slows the development of tumours [8], this fully agrees with observations discussed in this text, indicating that cutting the BCAA catabolism has anti-tumour effects.

AN OVERVIEW OF TUMOUR CELL METABOLISM

What is clear is that tumours display a hybrid metabolic rewiring, with phosphorylations of enzymes usually associated to catabolism and fasting, while their anabolism is activated by other phosphorylations of enzymes taking place when food is available. Normally, the endocrine pancreas senses the nutritional situation and selects through the release of catabolic or anabolic hormones the physiological status, these hormones activate signalling pathways that put a set of enzymes in a catabolic or anabolic configuration, by controlling their phosphorylation. In tumour cells, this mechanism seems defective [9]. One may hope that a nutritional reset, after fasting might repair the kinases or phosphatases that failed, and led to the hybrid catabolic / anabolic configuration of tumour cell enzymes. If this repair fails, tumour cells adapt to this situation by using, ketone bodies for making their mitochondrial acetyl-CoA. Ketones come either from fatty acid ketogenesis in liver, or from ketogenic BCAA amino acids transamination, the two supplies converge, for supporting the nutrition and development of tumour cells. The analysis of several works on tumours using SCOT and ketolysis or alternatively the BCAA transamination route to get their acetyl-CoA and citrate, indicates that the two pathways converge at the lipogenic-fatty acid synthesis route, receiving citrate from the two ketogenic sources. This does not exclude parallel effects of ketone bodies, on other targets and signalling routes in tumour cells: mTOR or MEK-ERK or AMP kinase pathways, or epigenetic changes induced by BHB inhibition of Histone deacetylase, favouring the expression of embryonic genes.

In sum BHB via SCOT-ACAT1 ketolysis and BCAA catabolism via transaminases and branched chain ketodehydrogenase complex (BCKDH), meet at ATP citrate lyase (ACLY) to feed the lipogenic demand of mitotic tumour cells.

The figure 1 presents the BCAA ketogenic amino acids pathway, and the BHB ketolytic pathway via SCOT and their junction at the lipogenic route in tumour cells. The ketone body dependency of tumour cells results from two glycolytic bottle necks between glycolysis and oxidative metabolism, while fatty acid synthesis turns-off the fatty acid degradation into acetyl- CoA. Hence, Ketones coming from liver ketogenesis or BCAA catabolism, become the essential source of mitochondrial acetyl-CoA supporting the citrate supply to fatty acid synthesis and lipogenesis. The regulation of these two ketone sources that tumours use for adapting their metabolism to the anabolic/ catabolic anomaly of their metabolism is regulated by a specific kinase (BCK) controlling the phosphorylation of the BCKDH on the one hand and ACLY plus FAS on the other hand, acting as a selection mechanism driving ketones from BCAA or SCOT, according to the available substrates, for feeding the lipogenic route where both

pathways converge. The kinase BCK kinase for BCAA ketoacid dehydrogenase (BCKDH) is activated by Src -Tyrosine phosphorylation (at Y246) [10]. It will then phosphorylate and turn-off BCKDH. However, if BCAA are available and transaminated, leucine gives alpha-ketoisocaproate, which inhibits the BCK kinase, leading to the dephosphorylation of BCKDC [11], which is subsequently turned ON, as indicated in Figure 1, triggering the beta oxidation of decarboxylated products of BCKDH. In the case of leucine and after several steps, acetyl-CoA and acetoacetate are generated; for isoleucine it gives acetyl-CoA and in fine succinyl CoA; while valine gives succinyl CoA, which enters the SCOT pathway. An essential point is that the BCK kinase Phosphorylates, and turns ON, ATP citrate lyase (ACLY) [12], and Fatty acid synthetase (FAS), activating the cytosolic lipogenic route, in parallel to its effect on BCKDH.

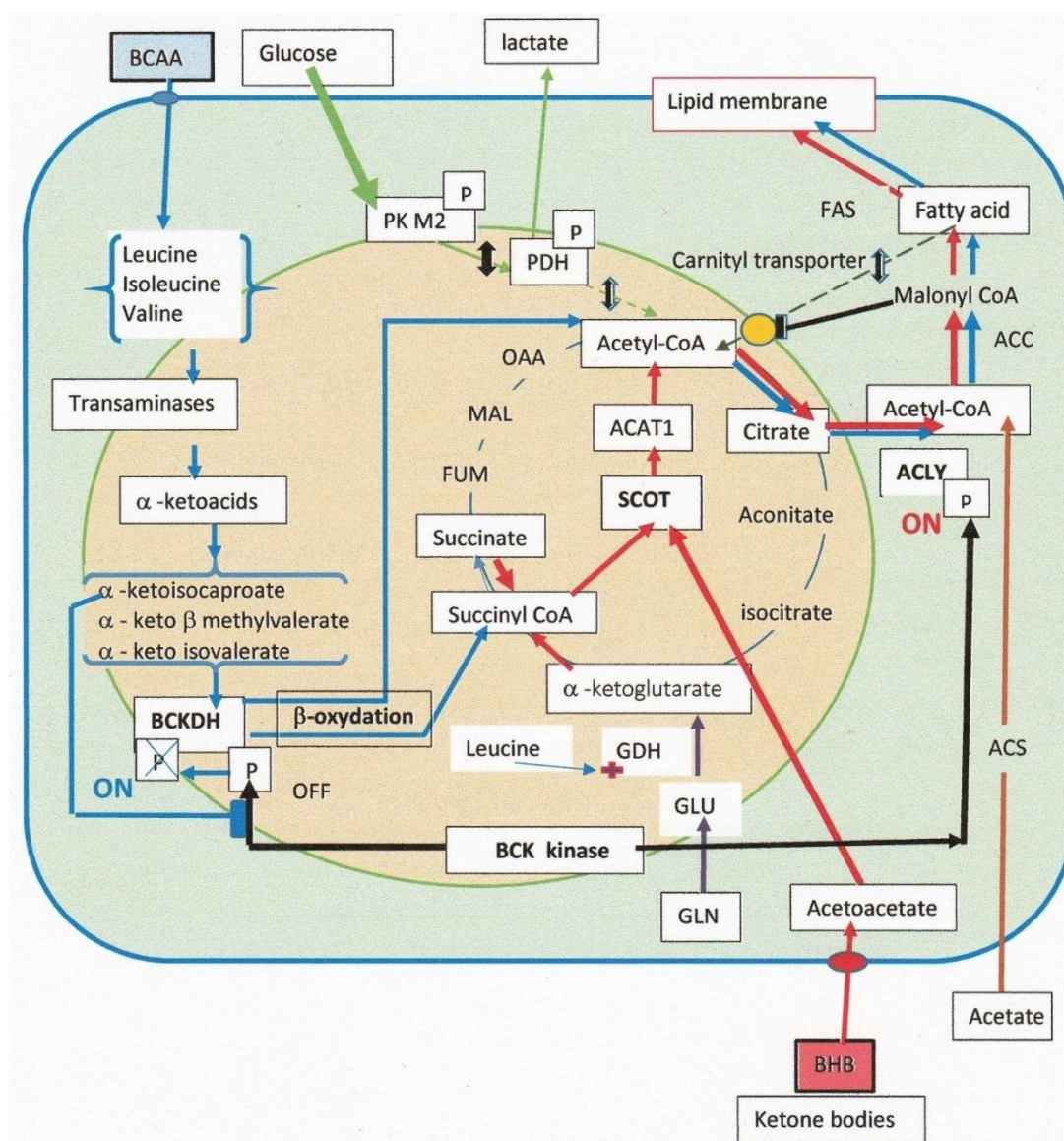


Figure 1: Branched chain amino acids and ketolysis support tumour growth

The figure recalls that the glycolytic supply of acetyl-CoA is interrupted by the phosphorylation of Pyruvate kinase (PK M2) and Pyruvate dehydrogenase (PDH) in tumour cells. In addition, fatty acid synthesis automatically turns off the fatty acid degradation into acetyl-CoA, since

malonyl CoA the product of acetyl CoA carboxylase (ACC) along the fatty acid synthesis route, closes the mitochondrial carnityl transporter of fatty acids and their degradation. Thus, tumour cells will produce acetyl-CoA by ketolysis of betahydroxyl butyrate (BHB) and acetoacetate, via a specific ketolytic enzyme, succinyl CoA: oxoacid-CoA transferase (SCOT) and acetoacetyl CoA transferase (ACAT1) (RED arrows). The acetyl- CoA then condenses with oxaloacetate (OAA), giving citrate, which exits the mitochondria and feeds lipogenesis, making lipid membranes for mitotic cells. In the cytosol ATP citrate lyase (ACLY) gives back acetyl-CoA, processed by ACC. Note the direct acetate entry converted into acetyl-CoA by acetyl-CoA synthetase (ACS).

Branched chain amino acids (BCAA) (Blue arrows) support the ketolytic dependent metabolism of tumours. Transamination of leucine, isoleucine and valine give the corresponding α keto acids, indicated. These are processed by branched chain α keto acid dehydrogenase (BCKDH). The products respectively derived from leucine, isoleucine and valine α keto acids, will then be β -oxidized to give in the case of leucine: acetyl-CoA and acetoacetate; in the case of isoleucine: acetyl-CoA and propionyl CoA; and in the case of valine: propionyl CoA. The propionyl CoA forms in fine succinyl-CoA. As illustrated, the acetyl-CoA and succinyl CoA enter in the Krebs cycle, and back-up the production of acetyl-CoA via SCOT, which favours tumour growth. The regulation of BCKDH is essential (black arrows). Indeed, a kinase (BCK) kinase, normally turns off BCKDH, but α ketoisocaproate the keto acid coming from leucine, selectively cancels this inhibition, reactivating BCKDH, and the supply of acetyl-CoA to the tumour. Other BCK kinase targets such as ACLY are activated by phosphorylation supporting tumour growth. BCK kinase activates MAP oncogenic signals. Note that leucine stimulates glutamate dehydrogenase (GDH) and the supply of α ketoglutarate from glutamate and glutamine.

Note in Figure 1 that Leucine stimulates Glutamate dehydrogenase, controlling the glutamine Glutamate entry; and that external acetate feeds directly the cytosolic acetyl-CoA synthesis.

KETOLYSIS via SCOT, OR BCAA CATABOLISM, SUSTAIN LIPOGENESIS AND TUMOUR PROGRESSION

A recent work on chondrosarcoma cells (CS) explored the expression of ketolytic enzymes; monocarboxylate transporter (MCT1) allowing the incorporation of BHB, the BHB dehydrogenase forming acetoacetate, then SCOT (the oxct1 gene product) forming acetoacetyl CoA, and ACAT1 leading to Acetyl-CoA. This was studied on starved and supplied (CS). The work concluded that that chondrosarcoma utilizes Ketone bodies for energy supply and proliferation, and indicates that the proliferation index increases for increasing doses of BHB. The authors concluded that BHB is a fuel source in CS [13].

We indeed observed a regression of tumours after SCOT and ketolysis inhibition, using the Lewis cancer model in mice [8].

In agreement with these observations, it was found in pancreatic ductal adenocarcinoma, that the uptake of BCAA was increased in tumour cells in comparison to control cells, the transaminase BCAT2 was also upregulated; knock down of the transaminase, impaired pancreatic ductal adenocarcinoma cell proliferation. This was also the case for the knock down of the branched chain keto acid dehydrogenase BCKDH, the next enzyme in the pathway.

The impaired proliferation following the knock down of BCKDH, was associated to a decrease in fatty acid synthesis [14]. These two examples indicate that two ketone sources are active in tumour cells: The BHB supply via SCOT-ACAT1 and the BCAA supply via transaminase and BCKDH, and converge at the fatty acid synthesis pathway. The upregulation of BCKDH was also observed in melanoma and shown to promote the expression of lipogenic enzymes ACLY and FAS [15].

In parallel observation on BCAA metabolism in cancer, an increased transaminase expression was found in myeloid leukaemia, induced by an oncogenic RNA binding protein (Musashi2), revealing that BCAA metabolism was activated and drives cancer progression in myeloid leukaemia [16].

In the same line, it was found that BCAA catabolism and BCAA transaminase promote the growth of breast cancer cells, and abolishment of BCAA catabolism by knocking down the transaminase inhibits cell growth and represses m-TOR-mediated mitochondrial biogenesis and function [17].

It was also shown that Leucine and BCAA catabolism contribute to the growth of bone sarcomas, by regulating AMP kinase and mTOR. In Chondrosarcoma, (SC) discussed above, the SCOT pathway was active and promoted proliferation. Osteosarcoma is more sensitive than chondrosarcoma to GABApentine a transaminase inhibitor. The lower transaminase in chondrosarcoma, presumably indicates that the SCOT pathway is more active for chondrosarcoma. In osteosarcoma, sensitive to GABApentine, the authors used an interesting leucine uptake inhibitor (N-acetyl leucine amide) to disrupt leucine metabolism in osteosarcoma cells and concluded that these cells depend of leucine catabolism for their energy and metabolic demands [18]. An interesting report on colorectal cancer indicates that the BCK kinase, which inhibits BCKDH, enhances the MAP kinase pathway by phosphorylating MEK, promoting transformation and tumorigenesis [19]. On the other hand the increase of BCAA transaminase in tumour cells forms alpha-ketoisocaproate, cancelling the inhibitory effect of the kinase on BCKDH, which activates BCAA catabolism. Moreover, in colorectal cancer, the SCOT- OXCT1 gene discovered from feces, correlated with cancer progression [20].

CONCLUSION

In fasting, catabolic hormones elicit the production of nutrients: glucose and ketone bodies, enzymes such PK or PDH, are inhibited by phosphorylation. When food becomes available, anabolic hormones should trigger the reactivation of these enzymes by de-phosphorylation. Tumour cells are unable to switch on and de-phosphorylate these enzymes. They consequently adapt their metabolism to this anomaly. Since mitotic cells have to synthesize fatty acids and new lipid membranes, they normally switch off, fatty acid degradation, and use ketone bodies to form acetyl-CoA and feed citrate in their lipogenic pathway. The ketones are metabolized via the SCOT pathway, or produced by the transamination of branched chain ketogenic amino acids. The two pathways converge at the lipogenic route. Other effects of intracellular ketones (BHB) re-express embryonic genes or activate NFkB transcription and inflammation; while extracellular ketones have an opposite action by activating the niacin receptor. Since ketones become an essential nutrient for tumour growth and progression in a cancer state, ketogenic diets might not be appropriate. Particularly if fasting and a nutritional reset fails to correct the abnormal phosphorylation of tumour cell glycolytic enzymes.

Whether the ketones come from the liver ketogenesis, and metabolized in tumours by the ketolytic SCOT pathway, or from branched chain amino-acids transamination. Inhibiting these two supplies would provide a complementary treatment to previously suggested compounds [5], [9] that selectively affected tumours and their metabolism. Further experimental studies are evidently required.

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