



Metabolic Pathways in Tumours: Vulnerable Steps for a Therapy

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Abstract: The adaptation of normal cells to physiological situations is fulfilled by metabolic changes that tumour cells have difficulty to perform. The enzymes involved being unable to take the adequate configuration. Thus, tumour cells rewire the system differently. They become dependent on ketolysis for producing acetyl-CoA and citrate in their Krebs cycle. Citrate feeds the cytosolic fatty acid synthesis pathway making lipid membranes for mitotic cells. In tumour cells, there are two bottle necks at the end of glycolysis, impairing the glycolytic supply of acetyl-CoA, while the synthesis of fatty acids, has turned off the degradation of fatty acid into acetyl-CoA. The shortage of acetyl-CoA for synthesizing citrate, is consequently compensated in tumours by a ketolytic acetyl-CoA supply. In addition, the exit of citrate in the cytosol, is supported by an inhibition of Aconitase, the second enzyme in the Krebs cycle. In fact, the circuits used by tumour cells gives them an advantage, they plunder glucose and ketone body stores. However, the rewired system, has vulnerable points that are discussed for finding a possible therapy.

Keywords: Cancer metabolism, ketones in cancer, SCOT, Aconitase, Siderophore.

INTRODUCTION

Many enzymatic properties and metabolic features of tumour cells have been compared to normal cells since the major findings of O. Warburg 1956 on the release of lactic acid by tumours [1], or the work of S. Mazurek and E. Eigenbrodt, on the M2 form of Pyruvate kinase (PK) in tumours [2] see review in 2003. In this context, studies on the effects of catabolic or anabolic hormones on enzymatic phosphorylation [3], revealed a mechanism adapting metabolic pathways to their physiological hormonal controls that rule: the synthesis of nutrients, the production of energy, or mitosis. In parallel works, the research on viral oncogenes, on cellular endogenous oncogenes, converged at common signalling pathways that were affected, In these pathways, anabolic insulin, or catabolic hormones had their specific receptor targets aimed by phosphorylating protein kinases and phosphatases, changing the phosphorylation of enzymes to be turned on or off, by phosphorylation [4]. We were in a way back to the physiological control of metabolic pathways, and to carbohydrate metabolism. In the present work, we compared the metabolic adaptation of normal cells and tumour cells with their enzymatic anomalies, in order to find out how they reached their physiological finalities. For example, how tumour cells rewire their metabolism if they fail to de-phosphorylate PK or Pyruvate dehydrogenase (PDH), when anabolic insulin is released, as if the phosphorylation of these enzymes triggered by catabolic glucagon or epinephrine for producing glucose and ketone bodies was maintained, in cells that receive an anabolic message for synthesizing fatty acids and lipids for making mitotic membranes. The metabolic adaptation of tumour cells will be reached, despite the enzymatic anomalies displayed, they even gain a metabolic advantage over normal cells, allowing their survival at the detriment of their host. Describing the metabolic circuit they have assembled might

help the identification of vulnerable points enabling one to fight the disease or perhaps to prevent it.

METABOLIC ADAPTATION OF NORMAL CELLS TO PHYSIOLOGICAL SITUATIONS

We first illustrate in Figure 1 the response of a normal cell, in diverse metabolic situations. In a first pathway, it displays a glycolytic metabolism continued by oxidative glycolysis, in a situation where the citrate condensation and the citrate efflux from mitochondria feeds the synthesis of fatty acids and lipids in the cytosol, for producing mitotic membranes. In this condition, malonyl CoA, the product of Acetyl-CoA carboxylase (ACC), along the fatty acid synthesis route, automatically closes the fatty acid degradation pathway, by inhibiting their mitochondrial transporter (carnityl-driven transporter) and the fatty acid entry in mitochondria. This stops their degradation by beta oxidation into acetyl- CoA. Hence, when fatty acid synthesis is turned ON, their degradation is turned OFF. The fatty acid supply of acetyl-CoA to Citrate synthetase (Cit.S) stops. In this first situation examined (citrate exit), the enzyme ATP citrate lyase (ACLY) in the cytosol, cleaves back citrate into acetyl-CoA feeding the lipogenic route, and oxaloacetate (OAA) processed by Malate dehydrogenase (MDH) forming NAD⁺, it is needed by Glyceraldehyde P dehydrogenase (GAPDH) in the glycolytic direction, ensuring the glycolytic supply of acetyl-CoA. The glycolytic source of acetyl-CoA is activated via PK and PDH that are consequently dephosphorylated. The next response examined, is a cell in which, citrate condensation is also turned on, but Aconitase the second enzyme of the Krebs cycle is activated. Hence, the citrate does not exit the mitochondria, the Krebs cycle turns and will build up the store of energy. The two entries of the respiratory chain are: Complex I, NADH dehydrogenase and Complex II: Succinate dehydrogenase (SDH) they decompose respectively NADH and FADH, the electrons are carried until oxygen via Complexes III and IV and reduce oxygen. While the protons, are ejected out of the internal mitochondrial membrane, for generating an external H⁺ gradient. Then protons flow back in, through the C1/Co ATPase, forming ATP (Chemiosmosis mechanism described by Mitchell). In these two situations, the citrate condensation reaction is ON. However, in this second situation the Krebs cycle turns, producing NADH, if the consummation of NADH by the respiratory chain is above its production. The energetic ATP store builds up. With the increase of the NADH /NAD ratio the condensation reaction slows down, reflecting the increase of ATP synthesis. In the first situation that we have examined, the exit of citrate does not feed the turning Krebs cycle, but the fatty acid synthesis pathway with acetyl-CoA, via the cytosolic cleavage of citrate, the other product formed (OAA), is processed by malate dehydrogenase giving NAD⁺ and malate. The NAD⁺ activates glycolysis at the GAPDH level, while malate forms pyruvate (malic enzyme). The pyruvate stimulates PDH or P.Carb glycolytic activities and the glycolytic supply of acetyl-CoA. If the glucose level and glycolysis decline, NAD⁺ and malate are in excess. Hence, malate starts entering in mitochondria through the Malate-alpha ketoglutarate shuttle, and produces (via MDH) OAA and NADH, The latter inhibits citrate synthesis, and the condensation reaction. Thus, the exit of citrate declines, the decrease of fatty acid synthesis and of malonyl CoA, opens back the fatty acid transporter, and the beta oxidation of fatty acid into acetyl-CoA, compensating the decline of the glycolytic acetyl-CoA source. Note that the glutamine supply forms glutamate, exchanged with aspartate by the corresponding shuttle. The glutamate, gives alpha ketoglutarate, which is exchanged for malate by the other shuttle.

In sum, the pathway supporting a citrate exit, opens the glycolytic acetyl-CoA supply, because the exit of citrate and fatty acid synthesis blocks the fatty acid source of acetyl-CoA. Reciprocally, when the glycolytic acetyl-CoA supply declines, GAPDH uses less NAD⁺. More NAD⁺ and associated malate are available. Consequently, the entry of malate through the shuttle increases and forms NADH, which inhibits the citrate condensation, and fatty acid synthesis. This opens back the fatty acid source of acetyl-CoA since the glycolytic one declined.

The third situation examined, is neoglucogenesis: Citrate synthetase, is turned off by an elevated NADH level, which spares OAA for neoglucogenesis, it is formed by pyruvate carboxylase (P.carb) in mitochondria, then PEPCK produces PEP in, then out of the mitochondria, PEP gives back OAA, then PEP again, to follow the neoglucogenic pathway until glucose. There is no possible return from PEP to pyruvate since PK will be inhibited by phosphorylation, avoiding a futile cycle. Not all cells produce glucose by neoglucogenesis, in response to catabolic hormones, they may also support their nutritional demand of glucose by glycogenolysis, or consume ketone bodies produced essentially in the liver by ketogenesis. Glucose, glutamine/glutamate transamination, ketolysis permit to compare the adaptability of normal and tumour cells to physiological situations. How would tumour cells react, if the de-phosphorylation of Pyruvate kinase PK, or Pyruvate dehydrogenase PDH, was not efficient, when switching from catabolic hormonal effects to anabolic insulin action?

TUMOUR CELLS REWIRE THEIR METABOLISM

In figure 2, a tumour cell adapts its metabolism to its physiological requirements, unlike normal stem cells that enter in mitosis to give two daughter cells with one that differentiates, the tumour cells gives two stem cells that both keep their mitotic project, a geometric increase of their number takes place. The tumour cells will then need to synthesise fatty acids and lipids for making mitotic membranes, they should follow as normal cells (in figure 1) a glycolytic metabolic pathway, continued by oxidative glycolysis. However, the PK and PDH steps remain inhibited by a phosphorylation, turning off the glycolytic source of acetyl-CoA. The citrate condensation reaction has then to find another source of acetyl- CoA, since the lipogenic pathway, via ACLY, and ACC forms malonyl CoA, which turns off the fatty acid supply of acetyl-CoA, while their synthesis operates. A mobilization of the ketolytic supply of acetyl- CoA via the specific ketolytic enzyme: succinyl CoA, oxoacid: CoA transferase (SCOT) [5, 6] becomes essential for tumour cells. Ketone bodies are formed in the liver, Beta hydroxybutyrate (BHB) is taken up by the cells, forms acetoacetate, and then acetoacetyl CoA via SCOT and acetyl-CoA via ACAT1 (thiolase). As hypoxic cells, tumour cells react by increasing the influx of glucose for overcoming their PK and PDH bottle necks, closing the entry in oxidative metabolism, It is a Pasteur effect, showing that yeast cells, consume more glucose in hypoxic conditions, Whereas, the conversion of the pyruvate formed after the first PK bottle neck, is converted into lactate (Warburg effect). Here, the two effects do not result from the lack of oxygen, but from the narrow communication between glycolysis and oxidative metabolism resulting from a maintained phosphorylation inhibiting PK and PDH. Hence, the cells that have to produce much citrate will use the ketolytic source of acetyl-CoA, via the specific ketolytic enzyme: (SCOT) [5, 6]. Citrate condensation depends also on oxaloacetate, and of the activity of PEPCK, P.Carb and MDH, including the malic enzyme, see figure 2. The efflux of citrate joins

the lipogenic route, the citrate exit is facilitated by an inhibition of Aconitase (presumably linked to an iron -sulphur cluster deficiency) or to a deficiency of their Frataxin carrier. Aconitase and isocitrate dehydrogenase have a lower activity, but the glutamine, and glutamate entry through the shuttle is fully active, glutamate is exchanged with aspartate. Glutamate then gives alpha ketoglutarate, which flows out through the second shuttle, and is exchanged with malate. The influx of malate forms OAA and NADH, which is actively consumed by the respiratory chain. The thickness of the arrows in figure 2, indicates that the pathway is boosted. Note that the transamination drives the shuttles and contribute to the release of lactate. The metabolic advantage gained by tumour cells supports their mitotic capabilities. The metabolic rewiring of their metabolism allows them to plunder body stores, glucose, glutamine, ketone bodies and ketogenic branched chain amino acids, these were studied in other works [7, 8]. The metabolic success of tumour cells, may eventually kill their host. However, Biochemists or Physiologists, may find a way to block the advantage gained by tumour cells and perhaps heal cancer, or decrease the carcinogenic probability.

CONCLUSION

The metabolic model presented for tumour cell metabolic rewiring considers the observations, of Warburg, Eigenbrodt and Mazurek or studies on the phosphorylation of PDH by PDH kinase, or our work on ketolysis that helped us to build this model [7, 8]. In addition, we earlier called attention on a possible role of the endocrine pancreas that would be unable to block catabolic glucagon when insulin release takes place, incriminating the role of the co-release of GABA or Somatostatin, inhibiting catabolism when anabolism is active. We considered that the hybrid metabolic rewiring observed in tumour cells, resulted from a desensitization of differentiated cells for insulin, while new stem cells with new insulin receptors, still not desensitized, responded to both anabolic insulin and catabolic glucagon, explaining the maintained phosphorylation of PK and PDH and hybrid response, while switching from catabolism to anabolism. We shall use the present model for explaining the carcinogenicity of several mutations and anomalies on enzymes of the Krebs cycle. First the SDH (complex II) mutations inhibiting this enzyme and causing tumours: Carney-Stratakis Syndrome. These are gastrointestinal tumours, pulmonary chondroma, paraganglioma, pheochromocytoma [9]. In The SDH deficient tumours, succinate is no longer converted into fumarate, and gives back succinyl CoA via succinyl CoA synthetase, which boosts the activity of SCOT and ketolysis, feeding tumour lipogenesis with acetyl-CoA. Compounds such as fenbendazole or albendazole and others are antiparasites in veterinary medicine, we proposed them in earlier works, as possible inhibitors of PEPCK and glucose metabolism. Their anticancer effect might result from an increase of SDH activity, counteracting the SDH deficient mutations [10]. The drug would then deprive SCOT of succinyl CoA, by increasing the conversion of succinate into fumarate. The subsequent decrease of SCOT activity decreases the acetyl-CoA supply to the lipogenic pathway of tumours, explaining the anticancer effect of the drug. A second example to consider is the isocitrate dehydrogenase mutations associated to gliomas [11]. The mutated enzyme forms an abnormal product: D-2-hydroxyglutarate, which increases the expression of branched chain amino acid transaminase. The metabolism of ketogenic amino acids should then increase, as well as the influx of leucine in the tumour cells. Leucine seems to inhibit the transaminase [12], but it is also an allosteric activator of Glutamate dehydrogenase, forming more alpha

ketoglutarate [13], which increases succinyl CoA, and SCOT activity, again the ketolytic supply of acetyl-CoA, stimulates the lipogenic pathway needed by mitotic cells favoring their development. The third example is linked to the exit of citrate from mitochondria in tumour cells. We have seen that aconitase activation by Sulphur- Iron clusters carried by Frataxin, allows the Krebs cycle to turn, decreasing the exit of citrate and its supply to the fatty acid synthesis route. The tumour cells favor the exit of citrate by having a low aconitase activity. There is a rare disease more frequent in Sweden: Plummer and Vinson syndrome, affecting women. A dysphagia, eventually leading to cancer is observed. It was found that an iron treatment, prevents the carcinogenic transformation [14]. Presumably, the activation of aconitase by the S-Fe clusters decreases the exit of citrate and decreases the lipogenic formation of mitotic membrane. Finally we know that an endogenous siderophore, 2-5 Dihydroxy benzoic acid (2,5DHBA), controls iron metabolism in erythropoiesis and iron recovery. The siderophore is produced by 3-OH butyrate dehydrogenase, (BDH2) a reductase involved in the conversion of BHB into acetoacetate. However, this first enzyme in ketolysis is a step that can be ensured by other reductases, followed by SCOT and ACAT1. Disruption of BDH2 by homologous recombination in mice, suppressed the formation of the siderophore, without affecting ketolysis. A microcytic anemia and iron accumulation in spleen is observed in BDH2 null mice. An exogenous supplementation of 2,5DHBA alleviates the splenic iron overload [15]. This siderophore may also be formed as a product of aspirin metabolism and flavonoid metabolism. Well if the siderophore activates aconitase by bringing to the enzyme the iron needed, one would then reduce the exit of citrate from mitochondria, and explain the anticancer effect, by a decrease of cytosolic lipogenesis needed for making mitotic cell membranes As might occurred in the case of Plummer and Vinson syndrome treated by iron complementation, or for the anticancer effects of aspirin and flavonoids [16].

Having reached the end of this presentation, we may propose to decrease the entry of ketone bodies in tumour cells, to decrease SCOT, to decrease the exit of citrate from mitochondria, and limit the synthesis of lipid membranes for mitotic cells. The best would be to prevent that cells rewire their metabolism as tumours do. The wrong road is at least indicated, avoiding it will require more experimental work. A final remark gives possible suggestions. Take a situation in which catabolic hormones are released during a fasting period, the phosphorylation of PK and PDH is normal in this condition. If food becomes available, these enzymes should be de-phosphorylated when anabolic hormones come on the stage. In cancer this does not to occur. Perhaps a fasting period followed by the intake of food, might reset the system, if it has the capacity to auto repair itself, during a "healthy resting period", in which one would delay the development of the ketolytic dependency. We would gain to know more about the reciprocal interactions between cells in the endocrine pancreas, the role of GABA co-released with insulin, or of somatostatin induced release elicited by SGLT2 a (sodium-glucose transporter)[17]. Particularly if they inhibit catabolism when anabolic insulin is released. How is this regulation affected in cancer, Is it possible that it triggers the rewiring process described?

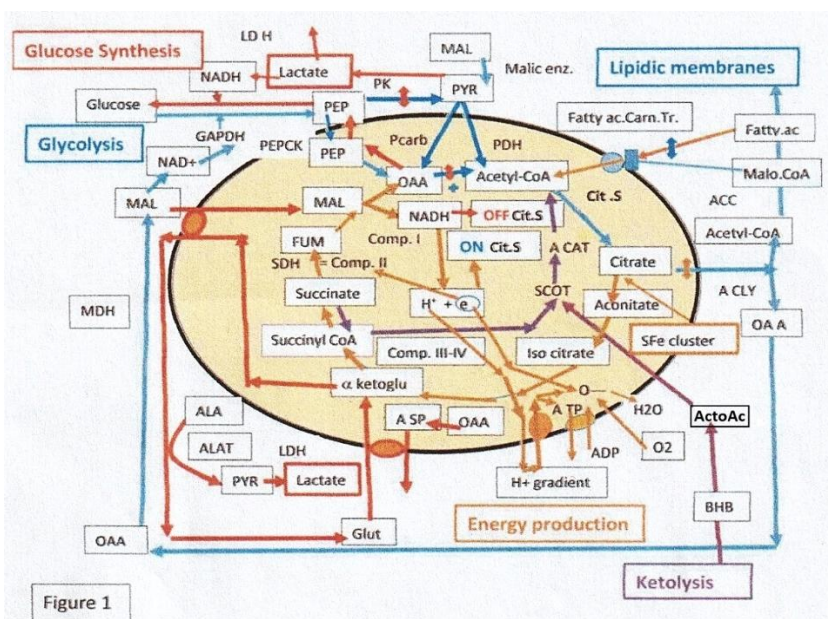


Figure 1: Mitochondrial metabolism in different physiological situations, (Top figure)

1: Synthesis of lipid membranes for mitotic cells requires fatty acid and lipid synthesis (Blue pathways). 2: Production of Energy (brown pathways) controlling the chemiosmosis mechanism synthesizing ATP, via the Proton gradient and electron fluxes carried by respiratory chain until oxygen. 3: The nutritional pathways (glycolysis in blue) glucose synthesis in red, and ketolysis in violet. Note that the mitochondrial shuttles driving the nutritional glutamine and glutamate influx are in red. One shuttle is a glutamate and aspartate antiporter and the other a malate and alpha ketoglutarate antiporter. The keys for explaining the circuits drawn are: Citrate condensation it is ON if NADH decreases in mitochondria, activating either the blue or brown pathways. The exit of citrate for the blue pathway means that aconitase is not activated by S-Fe iron clusters. The blue pathway feeds cytosolic lipogenesis with citrate. The cytosolic cleavage of citrate into OAA and acetyl-CoA means that acetyl-CoA via malonyl-CoA will close the fatty acid transporter and fatty acid degradation into acetyl-CoA, when the synthesis of fatty acids is turned on. While OAA via malate dehydrogenase forms NAD⁺, driving glycolysis at Glyceraldehyde P dehydrogenase. This ensures the glycolytic supply of acetyl-CoA. (It is associated to a de-phosphorylation of PK and PDH). The Brown pathway is On if both the citrate condensation and aconitase are ON, this stops the citrate exit, the Krebs cycle turns for making NADH and energy. The next key is that glucose synthesis is not the reverse of glycolysis. The enzyme Pyruvate kinase (PK) works only in the glycolytic direction. Two other enzymes will cover the passage from phosphoenol pyruvate (PEP) to Pyruvate. These are pyruvate carboxylase (in mitochondria) and PEP carboxykinase (in and out the mitochondria). In this situation, pyruvate forms OAA then PEP it exits and gives back PEP then OAA and again PEP until glucose. It is here necessary to close Pyruvate kinase by phosphorylation, avoiding a futile cycle, and the PDH entry in mitochondria. Thus, PDH is also phosphorylated and turned off. Note that transamination drives the shuttles in red, the glutamine glutamate nutritional input and the ketolytic input in violet.

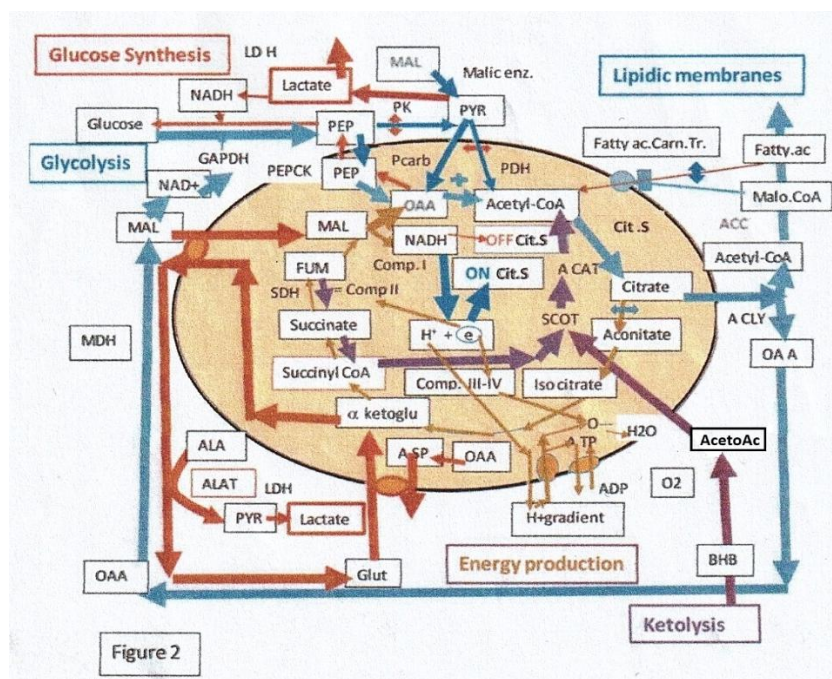


Figure 2: Metabolic perturbations of these pathways in Tumour cells

Glycolysis is elevated in tumour cells, However, PK its last step remains inhibited by phosphorylation, as occurs in neoglucogenesis (avoiding futile cycle). Moreover, PDH is inhibited by phosphorylation. The increase of the glucose influx tries to overcome the PK and PDH bottle necks explaining the Warburg and Pasteur effects in tumours. Hence, the glycolytic supply of acetyl- CoA is difficult. Since mitotic cells have to synthesize fatty acids and lipids for making membranes, there is an automatic inhibition of fatty acid degradation by malonyl CoA. In order to support the citrate condensation in their Krebs cycle, tumour cells have to open the ketolytic source of acetyl-CoA. The influx of BHB increases, forms acetoacetate. Succinyl CoA and acetoacetate are the two SCOT substrates, giving acetoacetyl CoA, converted into acetyl-CoA by ACAT1. The violet and blue pathways converge to form citrate. The latter might feed the turning cycle, but quits the mitochondria, since Aconitase is not activated. Iron metabolism, S-Fe clusters, are probably involved. Moreover, the efflux of citrate is supported by the decrease of NADH, boosting citrate synthase (compare the thickness of pathways in top and bottom figures)

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To Anita Birgitta Persson my wife, 1944-2026 in memoriam.