

# Tumor Cells are Vitally Dependent upon Ketolysis, Inhibition of Succinyl CoA: 3-Oxoacid-CoA Transferase Should Block Them

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## ABSTRACT

In tumor cells, ketolysis becomes the unique source of mitochondrial acetyl CoA. Indeed, the glycolytic acetyl CoA production is blocked (pyruvate kinase and pyruvate dehydrogenase are inhibited by phosphorylation). Whereas, the fatty acid degradation into acetyl CoA is also turned off by malonyl CoA, the product of acetyl CoA carboxylase, which forms with the synthesis of fatty acids, to automatically close down their degradation, by inhibiting the fatty acid mitochondrial transporter. Thus, inhibiting the ketolytic supply of acetyl CoA and the specific ketolytic enzyme: succinyl-CoA: 3-oxoacid-CoA transferase, should block the tumor. However, tumor cells are able to take-up acetate and convert it into acetyl CoA in their cytosol via an acetyl CoA synthetase and inhibiting this enzyme would make it difficult for tumor cells to survive.

**KEYWORDS:** Cancer metabolism; Ketone bodies; Succinyl-CoA: 3-Oxoacid-CoA transferase (SCOT); Inhibition of SCOT; Acetyl CoA synthetase inhibition; Cancer treatment

## INTRODUCTION

Insulin-Dependent Diabetes (type 1) may result from an attack of pancreatic beta cells by autoantibodies against the enzyme synthesizing GABA [1]. We know that pancreatic beta cells co-release insulin and GABA, with the insulin release correcting hyperglycemia, while GABA will act on GABA type A ionotropic receptors, inhibiting neighboring delta and alpha cells and respectively releasing somatostatin and glucagon. Hence, the release of anabolic insulin is associated with a mechanism switching-off via GABA, catabolic glucagon release, and somatostatin release [2,3]. Moreover, GABA acts on beta cell auto-receptors (metabotropic GABA type B) for putting an end to insulin release [4]. A GABA deficiency will then fail to completely turn off insulin release and the resulting leakage

of insulin will desensitize in the long-run insulin receptors in differentiated cells (an effect reminiscent of Type II diabetes or metabolic syndrome) [5]. The deficiency of GABA release also affects the mutual exclusion process of catabolism when anabolism takes place, sending a dual message to cells. Differentiated tissues resistant to insulin will take the catabolic part of this message and develop a neoglucogenic and ketogenic metabolism. Whereas, cells that are not resistant to insulin will receive both insulin and glucagon messages; these are new mitotic stem cells with new insulin receptors, not yet affected by the chronic desensitization provoked by insulin leakage. These cells will rewire their metabolism as one observes for tumor cells [6-10].

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## Metabolic Rewiring in Cancer, A Consequence of Mixed Pancreatic Signals

Tumor cells are avid for glucose and insulin elicits the incorporation of glucose transporters in their membrane. Indeed, following the binding of insulin to its tyrosine kinase receptor and the activation of MAP and PI3 kinases, a downstream effect stimulates a phospholipase forming Inositol 3, 4, 5 Phosphate (IP3) and Diacyl Glycerol (DAG). Then, an IP3-mediated calcium release from the reticulum in the cytosol triggers the exocytotic incorporation of glucose transporters in the cell membrane. In parallel, glycolysis increases due to the decline of cAMP, elicited by a calcium-activated phosphodiesterase, cancelling the inhibitory action of cAMP over fructose 2, 6 bis P synthesis, which increases this activator of glycolysis; incidentally, drugs (like rolipram) that inhibit the phosphodiesterase, would keep cAMP up and limit the glycolytic flux. In spite of the avid glucose influx, the phosphorylation of Pyruvate Kinase (PK) and Pyruvate Dehydrogenase (PDH), which inhibits these enzymes, closes the last steps of this increased glycolysis of tumor cells. In fact, tumor cells express the M2 form of PK that gives inactive dimers rather than active tetramers, forming a "bottleneck" at the end of the glycolytic pathway [11,12], which switches off the glycolytic production of acetyl CoA by PDH.

The PK bottleneck increases the influx of substrates into the pentose pathway, while Phosphoenolpyruvate (PEP) that has accumulated above the PK bottleneck will rather convert to Oxaloacetate (OAA) via PEP Carboxykinase (PEPCK). The question remains: What maintains this phosphorylation of PK and PDH? Presumably, the inhibitor CPI 17 of their phosphatase is synthesized via a stimulation of Protein Kinase C (PKC) by DAG [13]. We already know that PKC stimulates the production of this inhibitor, which cancels the calcium-dependent activation of the phosphatase PP1 by calcineurin, which normally inactivates another inhibitor, I1, of the PP1 phosphatase. The question then becomes, what keeps DAG elevated? Again, we already know that growth hormone stimulates Adipose Triglyceride Lipase (ATGL), which produces an excess of DAG. The stimulation of growth hormone is itself a consequence of the GABA deficiency, because it is not limited to the pancreas. Low GABA increases epinephrine release from the adrenals, which then inhibits somatostatin release. Consequently, growth hormone and insulin like growth factor increase. In this respect, ATGL, inhibitors would deserve a try for their potential therapeutic effect [14]. Inhibition of growth hormone, DAG and PKC should also be interesting (remember that DAG acts on PKC similarly to phorbol ester carcinogens).

In sum, the glycolytic source of acetyl CoA at the entry of the Krebs cycle is well closed. On the other hand, the insulin anabolic action boosts synthetic processes and fatty acid synthesis, needed for the making of new membranes for mitotic cells. In this pathway, malonyl CoA, the product of Acetyl CoA Carboxylase (ACC) at the beginning of the pathway, inhibits the transport and degradation of fatty acids in mitochondria, closing the beta-oxidation pathway, and the fatty acid source of acetyl CoA. Indeed, when fatty acid synthesis operates, the degradation of fatty acids automatically stops. Hence, if the two sources of mitochondrial acetyl CoA (glycolytic or fatty acid) are closed, mitotic stem cells will have to get their acetyl CoA from the ketolysis of ketone bodies coming from the liver or other tissues, selectively responding to catabolic hormones. Thus, with the acetyl CoA that comes from ketolysis and OAA coming from PEP, via PEPCK and other sources, the citrate condensation takes place, starting the Krebs cycle [15,16]. Citrate will then quit the

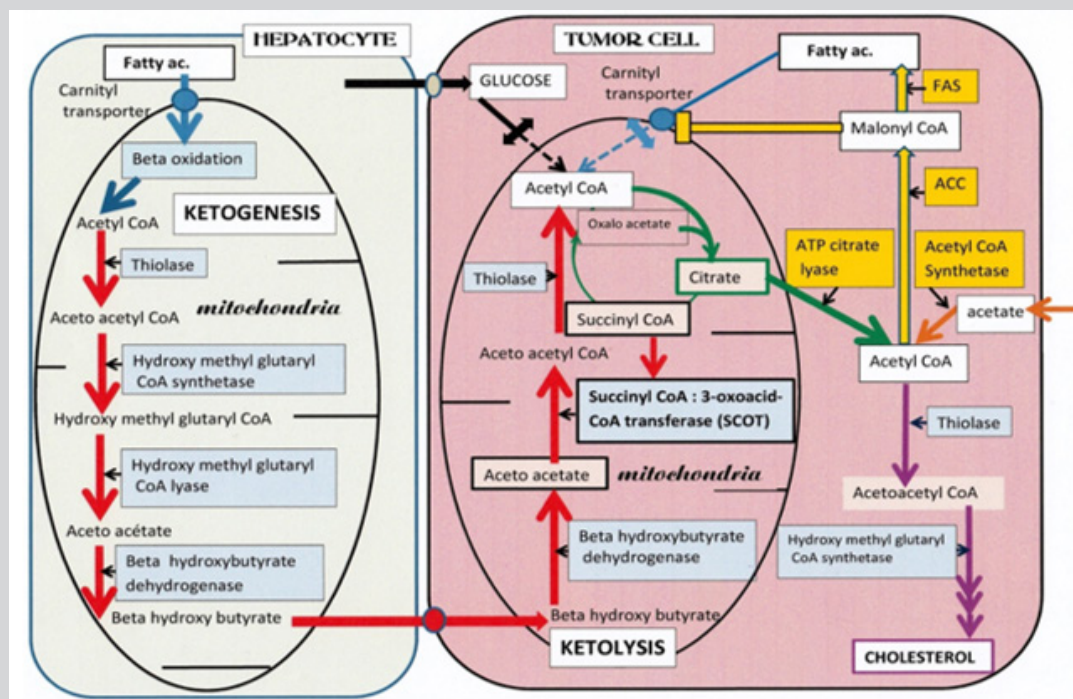
mitochondria and feed, via ATP citrate lyase, in the cytosol through the lipogenic pathway.

## Ketogenesis and Tumor Cell Ketolysis

We represent in Figure 1, a hepatocyte (left) responding to glucagon; it produces glucose and ketone bodies. The beta-oxidation of fatty acids is active. They enter the mitochondria via the fatty acid acyl carnitine transporter and produce intra-mitochondrial acetyl CoA. Then, four enzymes that support ketogenesis (indicated in the Figure 1) lead to beta hydroxybutyrate, after the reduction of acetoacetate; the latter also degrades into acetone and CO<sub>2</sub> (not represented). In catabolism, ketogenic amino acids, such as leucine and a few others, also feed the ketogenic pathway with acetyl CoA and acetoacetyl CoA, leading to acetoacetate and beta hydroxybutyrate. The released beta hydroxybutyrate enters the tumor cell through a transporter (Figure 1, right). It then reaches the mitochondria, where three enzymes (indicated in the Figure 1) will support ketolysis, resulting in acetyl CoA; the enzyme: Succinyl CoA: 3- Oxoacid-CoA Transferase (SCOT) represents the ketolysis specific step.

This ketolytic source of acetyl CoA is vital for tumor cells; indeed, the other sources of mitochondrial acetyl CoA, the glycolytic (via PK and PDH) or the beta-oxidation of fatty acids, are not working in tumor cells as above explained. Thus, only ketone bodies provide acetyl CoA (via SCOT) to the citrate condensation reaction that starts the Krebs cycle. The citrate condensation is particularly active; but, the citrate will quit the mitochondria and enter in the lipogenic pathway in the cytosol, forming fatty acids and lipids that are essential to build new membranes for mitotic cells. Thus, blocking SCOT, the citrate efflux and ATP citrate lyase, should hold back tumor development. A critique exists that SCOT inhibition "might come with all shades of grey", leaving a sufficient residual activity. We have anticipated this possibility by interrupting this supply of acetyl CoA in tumor cells, not only at the single SCOT, level but also on different enzymes in the lipogenic pathways. First, at the specific ketolytic enzyme SCOT, second the citrate condensation and citrate efflux from mitochondria, which feeds ATP citrate lyase to give back acetyl CoA in the cytosol. The addition of hydroxamic acid to already-tested lipoic acid and hydroxycitrate (that affect the citrate efflux and ATP citrate lyase) should greatly diminish the supply of acetyl CoA and improve the results. The cumulative effects of these compounds on the acetyl CoA formation in tumor cells might still be insufficient, since they develop a salvage pathway and they have in their cytosol an acetyl CoA synthetase capable of converting incorporated acetate into acetyl CoA, which then enters into the fatty acid synthesis and lipogenic pathways [17]. The acetyl and acetoacetyl CoA synthetases are enzymes forming adenylate intermediates before acetylation or acylation; inhibitors of these adenylating enzymes are available. Allicin or orotic acid are not particularly toxic and inhibit the salvage pathway from forming acetyl CoA in the cytosol. This concerted action on four enzymes should greatly affect the supply of acetyl CoA in tumor cells.

As Figure 1 indicates, there are still more enzymes that could be inhibited if necessary, the cytosolic thiolase, for example. Incidentally, we know that cholinergic neuromuscular synapses use this same acetyl CoA synthetase for making acetyl CoA, incorporating the precursor acetate in preference to pyruvate in the acetyl moiety of acetylcholine; whereas, brain cholinergic synapses use pyruvate rather than acetate as precursor for the acetyl moiety of acetylcholine [18].



**Figure 1:** Ketone bodies are vital for tumor cells. On the right, a tumor cell has a single ketolytic supply of mitochondrial acetyl CoA. Of the three enzymes that form the pathway, Succinyl-CoA: 3-Oxoacid-CoA Transferase (SCOT) is specific for ketolysis. Note that the glycolytic and fatty acid sources of acetyl CoA are both interrupted; on the glycolytic side, pyruvate kinase and pyruvate dehydrogenase are off in tumors, and on the fatty acid side, their degradation will stop since fatty acid synthesis is active. Indeed, the malonyl CoA intermediate closes down the carnityl transporter of fatty acids and beta-oxidation. The citrate efflux in the cytosol feeds ATP citrate lyase, giving back acetyl CoA and oxaloacetate. Acetyl CoA follows the synthesis pathway of fatty acids via Acetyl CoA Carboxylase (ACC) and Fatty Acid Synthetase (FAS). The ACC forms the malonate inhibitor of fatty acid degradation. Tumor cells possess an acetyl CoA synthetase that converts the exogenous acetate influx into acetyl CoA, supporting the lipogenic pathway and the synthesis of acetoacetyl CoA via a cytosolic thiolase. Tumor cells also have an acetoacetyl CoA synthetase feeding the cholesterol synthesis pathway. On the left, a differentiated cell (a hepatocyte) resistant to insulin but responding to catabolic glucagon produces glucose and ketone bodies. Fatty acids enter the mitochondria via their carnityl transporter and give acetyl CoA by beta-oxidation. The latter follows the ketogenic pathway, which consists of four enzymes, indicated in the figure. Ketogenesis forms acetoacetate and beta hydroxybutyrate. After release, beta hydroxybutyrate enters the tumor cell via a transporter and then in the tumor cell mitochondria and gives acetyl CoA by ketolysis. SCOT is the specific step supporting this vital supply of acetyl CoA to the tumor cell; it is its vulnerable point.

## The Ketogenic Diet Might not be a Good Idea

Initially, the ketogenic diet aimed to decrease the supply of glucose to tumors by replacing the glucose by ketone bodies, the other nutrient of cells, provided by a high-fat diet. However, in spite of several encouraging clinical reports, it seems that the ketogenic diet may not be a good idea, since ketone bodies are the only way for tumor cells to get their mitochondrial acetyl CoA. Several other works indicate that the utilization of ketone bodies drives tumor growth and metastasis [19,20]; this agrees with the present description indicating that tumor cell metabolism vitally depends on ketolysis for their mitochondrial acetyl CoA supply. It is thus "a priori" not indicated to follow a ketogenic diet.

It was then necessary to determine the clinical circumstances that introduced this diet. The recent publication of Klement [21] on the forgotten contribution of Wilhelm Brünings is in this respect particularly interesting. Following the discoveries of Warburg on tumor metabolism [22,23] (high glucose fermentation to lactate, in the presence of oxygen), Brünings proposed to decrease the glucose supply to tumors, using a low carbohydrate diet and insulin-induced hypoglycemia. However, it was necessary to feed patients with a

diet replacing the carbohydrates by lipids and proteins. Thus, the ketogenic diet was developed. The preliminary observations were encouraging; however, after several weeks, there was a rebound of the tumors and much disappointment (see the detailed description by Klement [21]).

What can we say about these trials? Evidently, in diabetes type I, the loss of beta cells and decrease of insulin release leads to hyperglycemia, with a loss of control over alpha cells that release glucagon, which elicits a ketogenic metabolism classically found in diabetes. On the contrary, when one injects insulin into an individual, there is a drop of blood glucose, since tissues take it up. Here, insulin counteracts the release of catabolic glucagon that normally elicits the production of glucose and ketone bodies. Hence, in Burning's trial, the initial effects of insulin-induced hypoglycemia must have been associated with a parallel decrease of ketogenesis, since insulin inhibits the alpha cell, via a mechanism that is not in this case GABA-mediated (we describe it later on in this review). The decrease of ketone bodies provoked by the insulin-mediated blockade of glucagon release in this initial phase of the trial coincided with the decrease in tumors' size. Indeed, without ketone



bodies, tumor cells could not make their mitochondrial acetyl CoA. However, with the gradual onset of the ketogenic diet, the supply of ketone bodies to the tumor starts again, and a rebound of the tumor sizes took place; the results of the trial were disappointing.

The direct effect of insulin on pancreatic cells has been recently unravelled [24]. Apparently, insulin acts on delta cells' insulin receptors, eliciting a glucose influx through glucose transporters and through the activation of an electrogenic co-transporter of glucose and sodium, the sodium-glucose luminal transporter 2 (known as SGLT2). The resultant increase of glycolysis forms ATP, which closes KATP channels. The overall membrane depolarization then opens calcium channels and triggers an influx of calcium, which induces, via ryanodine receptors, a further mobilization of calcium stores from the reticulum, activating the release of somatostatin from delta cells. The paracrine effect of somatostatin over neighboring cells is to inhibit both glucagon release from alpha cells and insulin release from beta cells. Thus, in relation to Brüning's trial, injected insulin, which elicits hypoglycemia, will directly stimulate delta cells' releasing somatostatin, which in turn inhibits the release of glucagon from alpha cells the counter-regulation by glucagon is thus switched off; neither glucose production nor ketogenesis take place after the insulin injection. Hence, the ketone bodies will decrease, depriving tumor cells of their vital acetyl CoA supply, in this initial phase of the trial tumor sizes' decrease. Later on, the ketogenic diet gradually provides ketone bodies to tumors and ketolysis feeds their mitochondria with a vital acetyl CoA supply, while the rebound of tumors takes place.

If Ketogenesis provides the acetoacetate substrate of Scot; the other substrate, succinyl CoA, comes via alpha ketoglutarate dehydrogenase, fed by glutaminolysis. Indeed, glutamate is shuttled in mitochondria, transaminates with oxaloacetate, to give alpha ketoglutarate, while aspartate shuttles out. The alpha ketoglutarate dehydrogenase then converts alpha ketoglutarate into succinyl CoA. In order to stabilize the supply of succinyl CoA to Scot, a mutation selects tumor cells displaying a succinohydrogenase deficiency. This mutation leaves more succinate to succinyl CoA synthetase, the preceding enzyme of the Krebs cycle, which then fuels more succinyl CoA to SCOT. This gain of function mutation for tumors appears in "Carney Triade" cancers. As for the other SCOT substrate, the ketogenic diet, abundantly provides it, for the benefit and survival of tumor cells that greatly dependent of ketolysis and SCOT to form mitochondrial acetyl CoA.

However, at an epigenetic level, beta Hydroxybutyrate Inhibits Histone Deacetylase (HDAC) and acetylated histones increase, possibly favoring the expression of genes that are silent in cancer, such as P53, which itself could be favorable if it occurs. On the other hand, we know that the butyrate inhibition of HDAC induces the expression of embryonic genes and proteins; it is the case for fetal hemoglobin or utrophine and others. Thus, the expression of embryonic M2 pyruvate kinase in tumor cells, instead of the M1 adult form might be a consequence of an increased supply of ketone bodies and HDAC inhibition; this is not favorable, since the M2 form of PK causes the glycolytic bottleneck in tumor cells. Note that the embryonic and adult PK proteins come from a different mRNA splicing of a single gene. Finding the right inhibitors and associated diet in cancer requires the advice of specialized nutritionists.

### SCOT Inhibition: A Possible Cancer Treatment

The therapeutic elements we propose in relation to this metabolic presentation have to go through tests on animal models before adding them to actual therapies. Moreover, even if the

compounds proposed come from published observations, they need an evaluation for the toxicity of mixtures (which could be different from the toxicity of each compound) prescribed for other indications.

In Figure 2, the numbers indicate a selection of enzymes to target. There are six points of attack that we have illustrated. The first target 'number 1' in Figure 2 is the ketolytic enzyme SCOT. The list of inhibitors found in the Brenda chemical database contains many compounds that are difficult to use or probably toxic, such as dinitrophenyl acetate, or 2, 2 difluorosuccinate [25]. Others could be tested, such as desulfo CoA, desulfopanteteine, acetylimidazole, 3 sulfopropanoate, N-acetylcysteamine, citrate, iodine and more. We then found, in the work of Picard and Jenks [26] on the active center of SCOT, a particularly interesting compound, acetoxyhydroxamic acid, which inhibits the enzyme-substrate complex. Acetoxyhydroxamic acid is available as tablets under the name of lithostat; it has been administered against kidney stones and bladder infections caused by bacteria raising the ammonia level in urine. Since SCOT inhibition by acetoxyhydroxamic acid is a central point in this review, we will now summarize the inhibitory mechanism studied by Pickart and Jencks [26].

In the active center of SCOT, the gamma carboxyl of a glutamyl residue reacts with the substrate succinyl CoA and forms a thioester bond with CoA in the enzyme-substrate complex. The thioester then transfers the CoA to acetoacetate. The inhibitor acetoxyhydroxamic acid inactivates the thioester enzyme-substrate complex, the most probable structure involving a bond between the enzyme carboxyl group and the hydroxylamine oxygen atom of acetoxyhydroxamic acid. The inhibition is partly reversible by increasing the CoA concentration. Acetoxyhydroxamic acid, which is a prescription for other medical indications, is less toxic than many inhibitors of SCOT found in the Brenda chemical base. Even if the inhibition is not total, it will limit the supply of acetyl CoA to the mitochondria in tumor cells and the citrate supply to the lipogenic pathway. Moreover, additional steps along this pathway can be blocked or diminished with other non-toxic compounds, as we did earlier and discuss below.

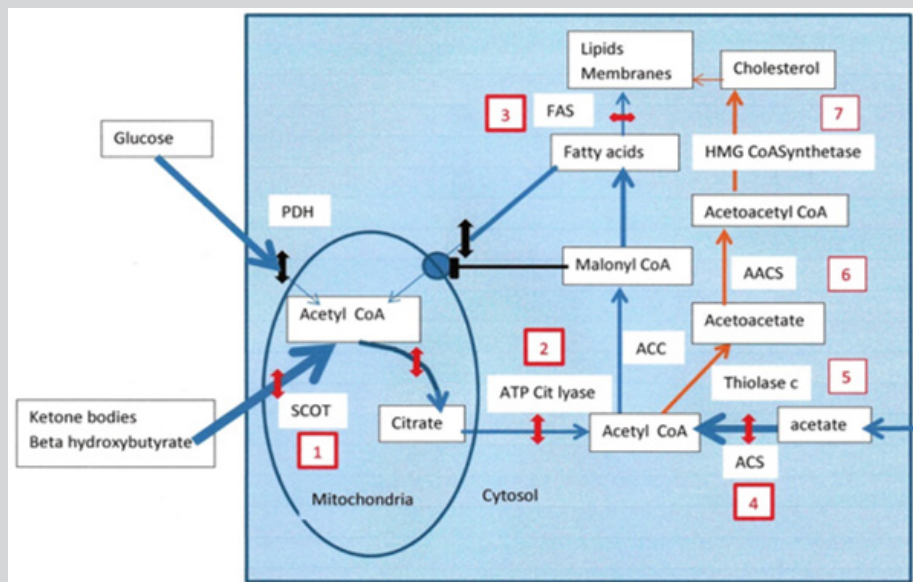
Salicylhydroxamic acid is another interesting compound to test on SCOT. Our literature search found that a class of HDAC inhibitors is the hydroxamic acid derivatives, such as suberoylanilide hydroxamic acid (known as SAHA), trichostatine, or vorinostat and others; these display clear anticancer properties [27]. However, we found no mention of a possible inhibition of SCOT in this observation, although this would explain the observed anticancer properties. The compound we preferably select for inhibiting SCOT is acetoxyhydroxamic acid; other derivatives (SAHA, vorinostat) deserve a try in spite of their action on HDAC.

The next target, 'number 2' in Figure 2 aims to decrease the efflux of citrate from the mitochondria to the cytosol, where citrate hydrolysis by ATP citrate lyase gives back acetyl CoA and OAA. Normally, the acetyl CoA produced by ATP citrate lyase feeds the lipogenic pathway, while OAA pushes the transamination chain, leading to pyruvate and lactate ultimately. In addition, OAA converts to malate, shuttles in mitochondria, and gives aspartate that enters into several pathways that are not the subject of the present work. We have previously shown that lipoic acid (the co-factor of PDH) associated with the ATP citrate lyase inhibitor hydroxycitrate, gave good results on animal tumors and in cancer [28].

We think that lipoic acid has in fact slowed down the citrate condensation reaction by reducing NAD into NADH, which inhibits

citrate synthetase; meanwhile, hydroxycitrate inhibits ATP citrate lyase. Another inhibitor of this step for study is bempedoic acid [29]. Presently, we would associate, as previously, hydroxycitrate lipoic acid and add the SCOT inhibitor. At the end of the lipogenic pathway, we find Fatty Acid Synthetase (FAS); this target is 'number 3' in Figure 2, and the known inhibitors of FAS are cerulenin,

C75, or orlistat. For these inhibitors and orlistat, we must await more information on side effects and toxicity. Blocking FAS would maintain the intermediate concentration of malonyl CoA that closes the beta-oxidation of fatty acid. While waiting on non-toxic inhibitors of FAS, drinking Green tea is an agreeable way to decrease FAS activity [30].



**Figure 2:** Enzymatic targets to stop the progression of tumors. Glycolytic and fatty acid supplies of mitochondrial acetyl CoA are not operational in tumor cells. Pyruvate Dehydrogenase (PDH) is switched off and malonyl CoA inhibits the fatty acid carnityl transporter (double black interruptions). The only supply of acetyl CoA comes from ketolysis via Succinyl-CoA: 3 Oxoacid-CoA Transferase (SCOT) 'target number 1'; its inhibition by aceto-hydroxamic acid or derivatives is represented by the red double arrow, as for the next targets. The citrate efflux and ATP citrate lyase is 'target 2', and previously tested compounds are lipoic acid and hydroxycitrate. At the end of the fatty acid synthesis pathway, 'target 3' is Fatty Acid Synthetase (FAS) and orlistat is an inhibitor but requires toxicity tests; green tea is more agreeable. 'Target 4' is Acetyl CoA Synthetase (ACS), and its inhibitors are allisine or orotic acid; others that we mention in the text are sulfonyl-adenosines, which inhibit Acetoacetyl CoA Synthetase (AACS) ('target 6') and also require toxicity tests. In between, 'target 5' is a cytosolic thiolase, and its inhibitors are trimetazidine and 4-pentenoic acid but they are difficult to handle. The last target is the enzyme 3-hydroxy-3-methyl glutaryl CoA synthetase ('target 7'); statins inhibit this enzyme and lower cholesterol but they each have side effects. In our opinion, targets 1, 2 and 4 are those we would handle first; with aceto-hydroxamic, hydroxycitrate and allisine or orotic acid, we would add lipoic acid if it does not reactivate SCOT.

In tumor cells, a salvage pathway in the cytosol supports the synthesis of acetyl CoA via an acetyl CoA synthetase that incorporates acetate taken-up by tumor cells, in acetyl CoA and in the lipogenic pathway; this target is 'number 4' in Figure 2. Acetyl CoA synthetase belongs to a class of enzymes forming adenylate intermediates, similar to acetoacetyl CoA synthetase, which is target 'number 6' in Figure 2, along the cholesterol synthesizing pathway. Inhibitors of adenylate-forming enzymes are sulfonyl-adenosines, celecoxib derivatives (AR12, AR14), adenosine 5'-ethyl phosphate, or a quinoxaline derivative [31-33,14]; however, these compounds need to be tested for their toxicity and side effects before being selected. We will here favor, in principle, non-toxic compounds, such as allisine from garlic that inhibits acetyl CoA synthetase [34]; it is also the case for an active substance from cow milk, orotic acid [35]. Orotic acid and allisin are already given for other indications. In between acetyl CoA synthetase (target 4) and acetoacetyl CoA synthetase (target 6), a cytosolic thiolase operates; this target is 'number 5' in Figure 2. Inhibitors of thiolase, such as trimetazidine (vastarel) or 4-pentenoic acid, are available but difficult to handle [36].

A compound such as dichloroacetate, which activates PDH by inhibiting PDH kinase, is difficult to use, and probably toxic. However, as shown by Madhok et al. [37], it induces apoptosis and cell cycle arrest in colorectal cancer cells. We simply want to point out that it displays, in parallel to this effect on PDK kinase, a blockade of ketolysis [38]. In the context of the present work, it is particularly interesting to underline this anti-tumoral effect. In addition, dichloroacetate will presumably inhibit acetyl CoA synthetase, which feeds acetate into the lipogenic pathway; this is an element of discussion on Ketolysis, provided by this laboratory reagent.

Finally, along the cholesterol synthesis pathway, statins that classically inhibit 3-hydroxy-3-methyl glutaryl CoA synthetase and cholesterol synthesis are difficult to handle because of their secondary undesired effects at the neuromuscular level; this target appears as 'number 7' in Figure 2. However, the side effects of statins require much attention before selecting them in this context.

In sum, a first possible choice for blocking the tumor could be aceto-hydroxamic acid or a derivative, plus hydroxycitrate and

lipic acid, plus allicine or orotic acid. We select these compounds because patients have taken them for other pathologies. However, to be cautious, one should test the mixture again. As for the other drugs with known side effects, such as statins, they need an evaluation in terms of risk on animal models as well. The drugs that we mention in the text (orlistat, dichloroacetate, and others) come from the biochemical and pharmacological literature. We have quoted their effect on enzymes and tumor cells because they validate the mechanism we discuss in this review. However, they are not in our selection for a possible therapy, precisely because we fear toxic or side effects. We do not exclude the compounds that we listed but we have to wait until their use extends beyond the biochemistry laboratories, to enter into pharmacology and toxicology study settings, which will take many years. We, therefore, limit our first selection to drugs that patients have taken already for other indications and to drugs without adverse effects. In addition, we still recommend testing the mixtures at the usual doses that one gives for their usual indication, on animal models first.

## CONCLUSION

For readers that do not have in mind the biochemical pathways and their regulation that we discuss in the present mini-review, we recommend the book by Elliott and Elliott [39], in which biochemical pathways are clearly illustrated and comprehensive in terms of nutrition, energy, and physiology. By inhibiting SCOT and the ketolytic source of acetyl CoA, one suppresses the only supply of acetyl CoA for tumor cell mitochondria. Indeed, the other sources of acetyl CoA, the glycolytic and the fatty acids are not operating in tumor cells, since PK and PDH are switched off, forming the glycolytic bottle neck, while the fatty acids degradation is also switched off when fatty acid synthesis is on. Hence, the blockade of SCOT deprives tumor cells of their vital acetyl CoA supply; they should then regress. In the cytosol of tumor cells, a direct supply of acetate can still form acetyl CoA via acetyl CoA synthetase and feed the lipogenic pathway. Inhibiting this entry as well would make it difficult for mitotic tumor cells to synthesize their membranes; they should then die or have to normalize their metabolism.

In fact, we do have evidence that SCOT inhibition by acetoxyhydroxamic acid or derivatives should provide a cancer treatment. This experimental evidence comes from the literature. Indeed, most acetoxyhydroxamic derivatives tried in another context for inhibiting HDAC display clear anticancer properties [27]. The researchers that tested hydroxamic acid derivatives for HDAC inhibition did not know, or did not mention, the earlier observation that hydroxamic acid was typically a SCOT inhibitor; proving the mechanism proposed in this minireview. Moreover, we realize that it is necessary to call attention to the ketogenic diet, often given to patients with the idea of compensating a decrease of the glucose supply. Apparently, patients seem to feel better initially but this may have harmful consequences, since tumor cells absolutely need ketone bodies to survive and develop, which explains the rebound of tumors observed by Brünings, who initially introduced the ketogenic diet, following an induced hypoglycemic trial by insulin [21].

In earlier works, we proposed to try to normalize cancer metabolism with different drugs. The aim was to control the starter mechanism that rewires metabolic pathways in cancer. This involves the pancreatic GABA trigger, the PK and PDH bottlenecks, the AMP kinase inhibition over acetyl CoA carboxylase and lipogenesis, and the growth hormone-ATGL-DAG-PKC axis [16]. Normalizing cancer metabolism requires an elevated number of compounds. We found some interesting minimal combinations of drugs [28] but much

work remains unfinished. The present proposal is different, since it takes advantage of the weak point of tumor cell metabolism, which vitally depends of SCOT and acetyl CoA synthetase. Their inhibition should hold back the progression of tumors, with a minimal number of compounds, giving a new possibility to add to present cancer treatments.

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