

## ATP-Citrate Lyase: A Key Player in Cancer Metabolism

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### Abstract

ATP-citrate lyase (ACLY) is a cytosolic enzyme that catalyzes the generation of acetyl CoA from citrate. Acetyl CoA is a vital building block for the endogenous biosynthesis of fatty acids and cholesterol and is involved in isoprenoid-based protein modifications. Acetyl CoA is also required for acetylation reactions that modify proteins, such as histone acetylation. ACLY is upregulated or activated in several types of cancers, and its inhibition is known to induce proliferation arrest in cancer cells both *in vitro* and *in vivo*. The present review highlights current knowledge about the role of ACLY in cancer cells, with special reference to the different pathways that are linked by ACLY. *Cancer Res*; 72(15); 3709–14. ©2012 AACR.

### Introduction

One of the most important and most common features of cancer cells is the dramatic reprogramming of their metabolic pathways (1). Because of the high proliferation rate of cancer cells, demand for energy and macromolecules is increased. To cope with these elevated requirements, cancer cells undergo major modifications in their metabolic pathways. One of the most important metabolic hallmarks of cancer cells is increased *de novo* lipid synthesis (2). Lipid synthesis pathways may include the fatty acid synthesis pathway as well as the mevalonate pathway, which leads to the synthesis of cholesterol and isoprenoids.

Various types of tumors display enhanced endogenous fatty acid biosynthesis, irrespective of levels of extracellular lipids (3). Most normal cells, even those with comparatively high proliferation rates, preferentially use dietary and/or exogenous lipids for synthesis of new structural lipids (2, 3). Some normal tissues also have a very active fatty acid synthesis pathway, such as adipocytes, hepatocytes, hormone-sensitive cells (4), and fetal lung tissue (5); however, in general, *de novo* fatty acid synthesis is suppressed in most normal cells. The upregulated fatty acid synthesis in cancer cells fuels membrane biogenesis in rapidly proliferating cancer cells and renders membrane lipid more saturated (6), thereby affecting fundamental cellular processes, including signal transduction, gene expression, ciliogenesis, and therapy response (6). The upregulated fatty acid synthesis in tumor cells is reflected by a substantial increase in expression and activity of various enzymes involved in the fatty acid synthesis pathway (3).

Several research groups have also associated the mevalonate pathway with cancer cell growth and transformation (7, 8). The first and rate-limiting step in this pathway is the formation of mevalonate by the enzyme 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR). Inhibition of HMGCR in normal cells triggers a robust homeostatic feedback response that ensures the cells upregulate and restore the mevalonate pathway (9). However, a number of tumors have been reported to have either deficient feedback control of HMGCR or increased HMGCR expression and activity (10).

ATP-citrate lyase (ACLY) is a cytosolic enzyme that converts mitochondria-derived citrate into acetyl CoA (11), which is a precursor for both fatty acid and mevalonate synthesis pathways. ACLY is reported to be upregulated in cancer cells, and its inhibition suppresses proliferation of certain types of tumor cells (12–14). This review focuses on current understanding of the role of ACLY in mediating tumor growth and its importance as a therapeutic target for cancer.

### ACLY Is a Cross-link between Glucose and/or Glutamine Metabolism and Fatty Acid Synthesis and/or Mevalonate Pathways

Many tumors display elevated aerobic glycolysis that correlates with increased tumor aggressiveness and poor patient prognosis (15). This enhanced glucose catabolism results in an excess of the glycolytic end product pyruvate. Although most of the pyruvate is converted into lactate and secreted out of the cells, part of the pyruvate enters the mitochondria. In the mitochondrial matrix, pyruvate is decarboxylated into acetyl CoA by pyruvate dehydrogenase (PDH; Fig. 1). The mitochondrial acetyl CoA combines with oxaloacetate (OAA) in a condensation reaction catalyzed by citrate synthase and results in generation of citrate that enters the tricarboxylic acid (TCA) cycle (16). Mitochondrial citrate can also be exported to the cytosol in the highly proliferating cells that use citrate as a biosynthetic precursor for lipogenic pathways. This withdrawal of citrate may stop the TCA cycle unless additional pathways are engaged to supply OAA to keep the

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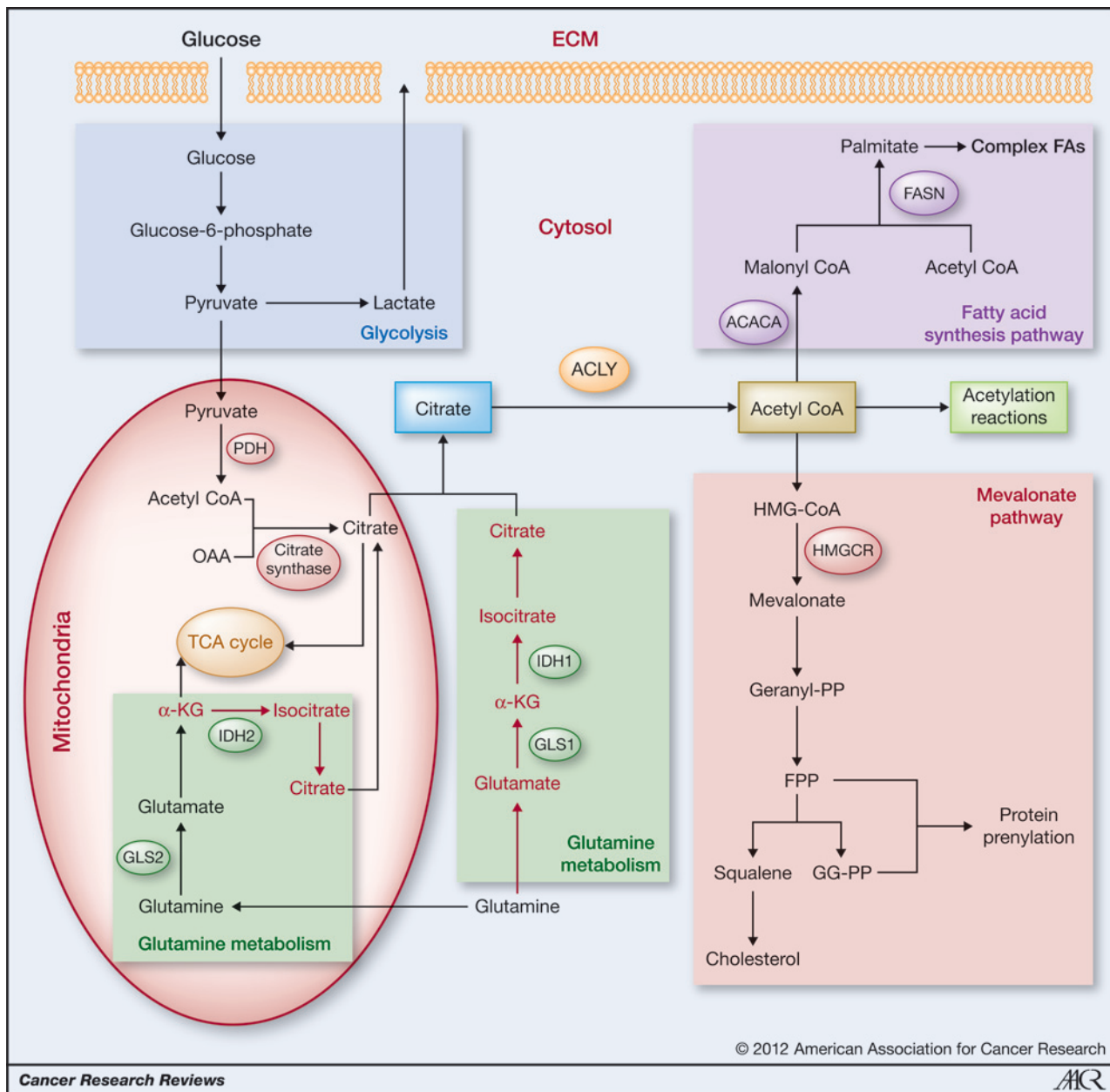


Figure 1. ACLY is a cross-link between glucose and/or glutamine metabolism and fatty acid synthesis and/or mevalonate pathways. When tumor cells that have functioning mitochondria are grown under normoxic conditions, the glycolytic pathway mainly provides citrate for acetyl-CoA production via an ACLY-catalyzed reaction. In tumor cells with defective mitochondria or in cancer cells proliferating under hypoxic conditions, reductive carboxylation of glutamine-derived  $\alpha$ -KG provides citrate for acetyl-CoA synthesis (red arrows). ECM, extracellular matrix; FA, fatty acid; FFP, farnesyl-pyrophosphate; geranyl-PP, geranyl-pyrophosphate; GLS1, glutaminase 1; GLS2, glutaminase 2.

cycle going. As mentioned above, condensation of OAA with acetyl CoA also forms the TCA cycle's canonical entry point. Hence, a constant supply of OAA is crucial for cancer cells. Although many cancer cells use glutamine metabolism for anaplerotic supply of OAA, some cancer cells use a compensatory anaplerotic mechanism in which pyruvate is carboxylated to form OAA in a reaction catalyzed by pyruvate carboxylase (17).

Earlier, it was thought that glucose metabolism was the main source of citrate for the downstream pathways. How-

ever, according to recent reports, in tumor cells with defective mitochondria or in proliferating cells under hypoxic conditions, reductive carboxylation of glutamine-derived  $\alpha$ -ketoglutarate ( $\alpha$ -KG) is responsible for supplying citrate for *de novo* lipogenesis (Fig. 1; refs. 18, 19). This pathway uses mitochondrial and cytosolic isoforms of isocitrate dehydrogenase (IDH).

In the cytoplasm, citrate is converted into acetyl CoA by ACLY. Acetyl CoA is an essential substrate for fatty acid synthesis and mevalonate pathways (12).

In the fatty acid synthesis pathway, acetyl CoA is carboxylated to malonyl CoA by acetyl-CoA carboxylase (ACACA). Next, the main lipogenic enzyme, fatty acid synthase (FASN), carries out the condensation of acetyl CoA and malonyl CoA to produce the long-chain fatty acid palmitate (Fig. 1; ref. 3). Acetyl CoA is also a precursor for the mevalonate pathway. As shown in Fig. 1, this pathway leads to the synthesis of farnesyl-pyrophosphate (FPP), which is the branch point for several pathways. In addition to participation in cholesterol biosynthesis, FPP can be converted into geranylgeranyl-pyrophosphate (GG-PP). Both FPP and GG-PP are, respectively, involved in farnesylation and geranylgeranylation of a variety of proteins (Fig. 1). Farnesylation and geranylgeranylation, together termed as prenylation, are required for the ability of Ras and Rho proteins to induce malignant transformation, invasion, and metastasis (20, 21).

Moreover, acetyl CoA is required for acetylation reactions, for instance, histone acetylation, which modify proteins having critical roles in regulating global chromatin architecture and gene transcription (22).

### Deregulation of ACLY in Cancer Cells

Distinctive elevation of ACLY expression and activity has been reported in lung, prostate, bladder, breast, liver, stomach, and colon tumors (13, 23–26). In human lung adenocarcinoma, the expression of phosphorylated ACLY was correlated with stage, differentiation grade, and poor prognosis (13). Thus, overexpression and activation of ACLY were found to be a statistically significant negative prognostic factor for at least this type of cancer (13).

The phosphoinositide 3-kinase (PI3K)/Akt pathway is a critical regulator of cell survival in several types of malignancies. In cancer cells, the phosphorylation and activation of ACLY are reported to be directly regulated by Akt (13). Akt also upregulates ACLY mRNA levels via activation of SREBP-1, a transcription factor for genes involved in cholesterol and fatty acid synthesis (27). However, ACLY protein levels are independent of SREBP-1 (13). It has been suggested that the PI3K/Akt pathway stimulates ACLY activity predominantly through phosphorylation of ACLY rather than transcriptional upregulation. This phosphorylation of ACLY contributes to its protein stabilization (13). ACLY can be phosphorylated at different sites by other kinases, such as nucleoside diphosphate kinase (28) and cyclic AMP-dependent protein kinase (29). It has also been shown that treatment with PI3K inhibitors does not have a dramatic effect on dephosphorylation and inactivation of ACLY in lung cancer cells. Therefore, it has been suggested that ACLY activity in cancer cells is also regulated by some other pathways (13).

### ACLY Inhibition for Cancer Therapeutics

The elevation of ACLY activity and expression status in cancer cells suggests that ACLY inhibition may be an attractive approach for cancer therapy. Various inhibitors of ACLY have been evaluated, mostly for their ability to block fatty acid or cholesterol biosynthesis. However, little emphasis

has been placed on their potential role as antitumor drugs. A number of citrate analogues were studied as ACLY inhibitors. These analogues included (+) and (–)-2,2-difluorocitrate, both of which showed activity against rat liver ACLY (30). In addition to the synthetic inhibitors, a naturally occurring citrate analog, namely (–)-hydroxycitrate, was found to be a potent inhibitor of ACLY (30). Treatment with (–)-hydroxycitrate results in decreased cholesterol and fatty acid synthesis in HepG2 cells. However, this inhibitor has certain limitations; for example, it is inefficiently transported across the cell membrane, and very high concentrations are required to achieve complete inhibition of ACLY activity (30). Moreover, (–)-hydroxycitrate inhibits IDH at concentrations that are similar to those required to inhibit ACLY. Another ACLY inhibitor shown to have antiproliferative effects on lung cancer cells, both *in vivo* and *in vitro*, is SB-204990 (12). Radicol, a naturally occurring antifungal macrolide, noncompetitively inhibits rat liver ACLY. However, it has been much more widely studied for its ability to bind to and inhibit Hsp90. Although several ACLY inhibitors have been described, at this point they only have a heuristic value. The therapeutic potential of ACLY inhibitors needs further clarification. Additional studies are required to verify the sensitivity and specificity of existing inhibitors and to identify more potent and specific ACLY inhibitors.

Many research groups are using RNA interference (RNAi) knockdown of ACLY to study the antiproliferative effects of ACLY inhibition in cancer cells. Inhibition of ACLY by either RNAi or pharmacologic inhibitors results in growth arrest in tumor cells, both *in vitro* and *in vivo* (12, 13). The antiproliferative effects of ACLY suppression are mediated by cell-cycle arrest (12, 13) and induction of apoptosis (31).

Downregulation of ACLY in implanted lung adenocarcinoma in mice not only caused tumor suppression but also induced glandular differentiation (12, 13). It was shown that the implanted tumors were poorly differentiated and exhibited a disorganized cellular architecture. In contrast, the tumor tissues in which ACLY was knocked down displayed a more differentiated morphology, closer to that of the normal lung tissue. This differentiated morphology was marked by the presence of glandular structures bearing central lumens (12). Notably, the loss of phosphorylated ACLY was confined to the glandular structures (13). Mucin production, the characteristic of differentiated respiratory epithelium, was only detected in tumors in which ACLY was downregulated (12). The chronic myelogenous leukemia cell line K562 was also shown to undergo erythroid differentiation in response to ACLY inhibition (12). This effect was comparable with the erythroid differentiation induced by the Hsp90 inhibitor radicol, which has been promoted as a therapeutic agent on the basis of its ability to induce erythroid differentiation in this tumor model.

An interesting speculation made by Hatzivassiliou and colleagues was that the proliferation arrest induced by ACLY inhibition correlates with the glycolytic phenotype of the tumor (12). Cancer cells displaying a high rate of glucose metabolism are more severely affected by ACLY inhibition, whereas those displaying a low rate of aerobic glycolysis are



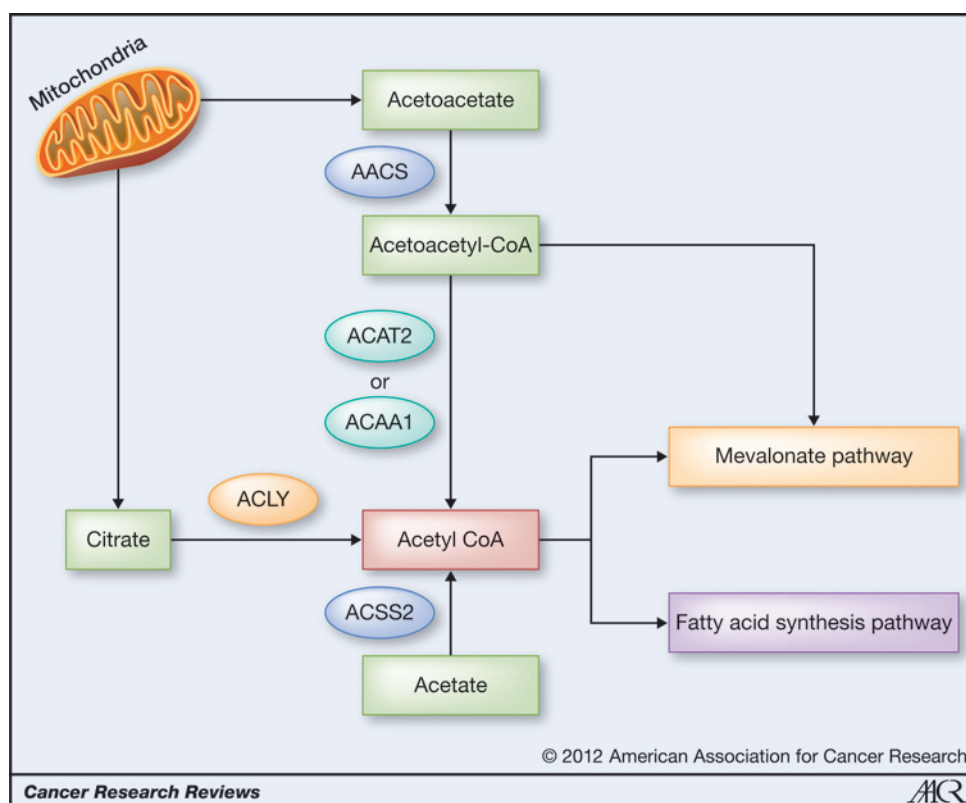


Figure 2. Different pathways for acetyl-CoA synthesis. ACLY is not the only source of acetyl CoA in cells. The cytosolic enzyme ACSS2 also produces acetyl CoA using acetate as a substrate. In liver cells, ketogenesis supplies acyl CoA chains to the lipogenic pathways. Acetoacetate produced by ketogenesis in mitochondria can be exported to the cytosol where acetoacetyl-CoA synthetase can catalyze the first step in a series of reactions that form short-chain acyl CoAs and lipids. AACs, acetoacetyl-CoA synthetase; ACAA1, acetyl-CoA acyltransferase 1; ACAT2, acetyl-CoA acyltransferase 2.

largely unaffected (12). Hence, therapeutic strategies targeting ACLY should take the glycolytic status of the cells into consideration.

### Proliferation Arrest Induced by ACLY Inhibition in Cancer Cells: Proposed Mechanisms

As mentioned earlier, ACLY catalyzes generation of acetyl CoA, which is a vital building block for the endogenous biosynthesis of fatty acids and cholesterol and is also involved in isoprenoid-based protein modifications. Hence, the tumoricidal effects that occur after ACLY blockade may relate to the cellular starvation of the end products of any of these aforementioned pathways.

It was previously established that inhibition of genes involved in fatty acid synthesis, such as FASN and ACACA, induces apoptosis in cancer cells. This apoptotic effect is rescued by fatty acid supplementation (3). Therefore, it could be hypothesized that if ACLY-silencing-induced proliferation arrest is due to fatty acid starvation, then fatty acid supplementation will also rescue the cells from these antiproliferative effects. Migita and colleagues reported that fatty acid supplementation does not rescue lung cancer-derived A549 cells from the proliferation arrest mediated by ACLY knockdown (13). However, we observed that, in certain cell lines, ACLY blockade-induced proliferation arrest could be rescued by fatty acid supplementation (N. Zaidi, J.V. Swinnen, and K. Smans; unpublished data).

ACLY inhibition can also affect the mevalonate pathway. Therefore, it can be speculated that tumoricidal effects of ACLY

blockade are caused by the shortage of cholesterol or isoprenoid. Recently, it was reported that the antitumor effects of ACLY inhibition are dramatically enhanced in combination with statins, the cholesterol-lowering drugs that inhibit HMGCR (31). ACLY deficiency was shown to intercept the AKT signaling pathway (31). Moreover, statin treatment of ACLY-deficient cells resulted in further decrease in AKT activation, together with downregulation of the mitogen-activated protein kinase pathway. This effect might be due to systemic cholesterol lowering that may interfere with cell growth via the impairment of cell membrane synthesis. The importance of the cholesterol synthesis pathway in ACLY knockdown-induced proliferation arrest needs further elucidation. Moreover, the effect of ACLY downregulation on isoprenylation, which includes farnesylation and geranylgeranylation, needs further clarification.

As ACLY uses citrate as a substrate, the absence of ACLY may result in accumulation of cytosolic or mitochondrial citrate, resulting in the impairment of glycolysis and/or mitochondrial function. Hanai and colleagues reported that ACLY knockdown-induced inactivation of the AKT-signaling pathway was augmented by addition of exogenous citrate (31). The ACLY-silencing-induced antiproliferative and apoptotic effects were further enhanced by citrate supplementation (31).

ACLY is also involved in determining the total amount of histone acetylation in multiple mammalian cells (22). Although histone acetylation is critical in regulating global chromatin architecture and gene transcription, ACLY-dependent

acetylation is thought to contribute to the selective regulation of genes involved in glucose metabolism. The expression of insulin-responsive glucose transporter, Glut4, as well as 3 key regulators of glycolysis, hexokinase-2, phosphofructokinase-1, and lactate dehydrogenase A, are all reported to be substantially suppressed upon ACLY silencing (22). Hence, the significance of ACLY in histone acetylation in cancer cells is also an important question for future studies.

### ACLY-Independent Pathways Can Also Provide Acetyl CoA to Lipogenic Pathways and May Influence the Sensitivity and/or Insensitivity to ACLY Knockdown

It is important to consider that ACLY might not be the only source of acetyl CoA in tumor cells. The cytosolic enzyme acyl-CoA synthetase short-chain family member 2 (ACSS2) produces acetyl CoA using acetate as a substrate (Fig. 2; ref. 32). Mammalian cells mainly use glucose as their major carbon source and are exposed to only low concentrations of extracellular acetate. Moreover, conversion from acetate to acetyl CoA is an energy-dependent process, whereas ACLY production of acetyl CoA from glucose is an energy-producing reaction.

Nevertheless, upon ACLY silencing, ACSS2 may compensate for the loss of ACLY depending on the availability of acetate. It was reported that incubation of cells with supra-physiologic levels of acetate rescues cells from proliferation arrest or decreased histone acetylation induced by ACLY suppression (22, 31). As mentioned above, the ability of ACLY downregulation to induce proliferation arrest is associated with the glycolytic phenotype of the tumor (12). The cells with a high rate of aerobic glycolysis are vastly reliant on glycolytic carbons for lipid synthesis. Consequently, in these cell lines, ACLY blockade causes substantial proliferation arrest along with decline in glucose-dependent lipid synthesis, whereas acetate-dependent lipid synthesis

remains unaffected (12, 13). However, in some cell lines with a low-glycolysis phenotype, ACSS2 seems to be important for acetate-dependent lipid synthesis and growth of cancer cells (33).

In liver cells, ketogenesis supplies acyl-CoA chains to the lipogenic pathways. In this pathway, mitochondrial acetyl CoA formed in the pyruvate dehydrogenase complex reaction is converted into acetoacetate after a series of reactions taking place in the mitochondria (16). This acetoacetate can be exported from the mitochondria to the cytosol, where acetoacetyl-CoA synthetase can catalyze the first step in a series of reactions that form short-chain acyl CoAs and lipids (Fig. 2; ref. 16). Although this pathway is mainly active in liver cells, its relevance in cancer cells upon ACLY knockdown is unclear and poses an interesting question for future studies.

### Conclusions

Increasing evidence supports the importance of ACLY in tumor cell growth. The fact that ACLY is upregulated in several types of cancer cells and that, upon inhibition of ACLY, cancer cells undergo a proliferation arrest both *in vivo* and *in vitro* indicates that this enzyme plays an important role in cancer cell progression. ACLY is involved in several pathways, and it is important to elucidate which of these pathways is the most relevant driver of its oncogenic potential to understand the possible benefit of ACLY inhibition. ACLY inhibition may provide a potential advantage over therapeutic strategies targeting other lipogenic enzymes, because ACLY is located upstream of the other lipogenic enzymes.

### Disclosure of Potential Conflicts of Interest

J.V. Swinnen received commercial research grants from and is a consultant to Janssen Pharmaceutica. No potential conflicts of interest were disclosed by the other authors.

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