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REVIEW

The complexity of the serine glycine one-carbon pathway in cancer

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The serine glycine and one-carbon pathway (SGOCP) is a crucially important metabolic network for tumorigenesis, of unanticipated complexity, and with implications in the clinic. Solving how this network is regulated is key to understanding the underlying mechanisms of tumor heterogeneity and therapy resistance. Here, we review its role in cancer by focusing on key enzymes with tumor-promoting functions and important products of the SGOCP that are of physiological relevance for tumorigenesis. We discuss the regulatory mechanisms that coordinate the metabolic flux through the SGOCP and their deregulation, as well as how the actions of this metabolic network affect other cells in the tumor microenvironment, including endothelial and immune cells.

Introduction

Altered cellular metabolism is a universal feature of human tumors, the effects of which extend beyond deregulated cellular energetics and encompass most of the hallmarks of cancer (Vander Heiden and DeBerardinis, 2017). Ongoing studies are revealing the metabolic complexity of tumor cells and a roadmap of the metabolic alterations that are of potential clinical relevance (Goveia et al., 2016; Hakimi et al., 2016; Hu et al., 2013; Peng et al., 2018; Reznik et al., 2018). They are also revealing common patterns of cancer metabolic reprogramming, despite the highly heterogeneous levels of metabolic enzymes and metabolite abundance across tumor types (Hu et al., 2013; Peng et al., 2018; Reznik et al., 2018).

The serine glycine and one-carbon pathway (SGOCP) is a metabolic network recurrently up-regulated in tumors and of high clinical relevance (Ducker and Rabinowitz, 2017; Locasale, 2013; Mehrmohamadi et al., 2014; Nilsson et al., 2014; Yang and Vousden, 2016; Zhang et al., 2012). The core of this pathway consists of two interconnected cycles: the folate and the methionine cycles. The SGOCP utilizes 5-methyl-tetrahydrofolate, a diet-derived cofactor, as a scaffold to transport one-carbon units donated by the interconversion of serine to glycine. Serine, as its major one-carbon donor, is the central amino acid in the SGOCP. Thus, serine availability, via its extracellular uptake or de novo synthesis, plays a decisive role in controlling the SGOCP's activity and function (Locasale, 2013; Yang and Vousden, 2016). However, additional enzymatic reactions from salvage pathways and the catabolism of other amino acids can replace serine as the obligate one-carbon unit donor. This alternative source of one-carbon units becomes particularly relevant when serine availability is limited. The outputs of the SGOCP include key metabolites that maintain the biosynthesis of nucleotides, proteins, and lipids; it also supports redox metabolism and fuels the methyltransferase reactions that shape the epigenetic landscape. Here, we review recent findings that have uncovered crucial roles for the SGOCP in tumorigenesis, with a particular focus on the biological regulatory mechanisms and the clinical relevance of the SGOCP activity in cancer and nontumor cells.

The SGOCP in tumors

Tumor-promoting actions of the SGOCP

The SGOCP is a set of metabolic networks organized around a core of two almost identical, intertwined cycles of methylation, in the cytoplasm and mitochondria, which use folate derivatives as carriers (Fig. 1). This coordinated division of labor maintains two compartmentalized pools of metabolic intermediates. Several additional metabolic pathways support the uptake, processing, and incorporation of metabolites (inputs) that feed into these two core reactions (Fig. 1 A). Additional metabolic reactions control the recycling of intermediary metabolites and replenish and coordinate the mitochondrial and cytoplasmic pools of folate species (Fig. 1 A). Catabolic reactions and salvage pathways also repurpose and/or detoxify metabolic byproducts and act as alternative sources of one-carbon units that can replace serine as the main one-carbon donor (Fig. 1 A). Finally, the existence of additional modules in this complex metabolic network add failsafe mechanisms and provide redundancy and detoxifying pathways to avoid the buildup of toxic byproducts

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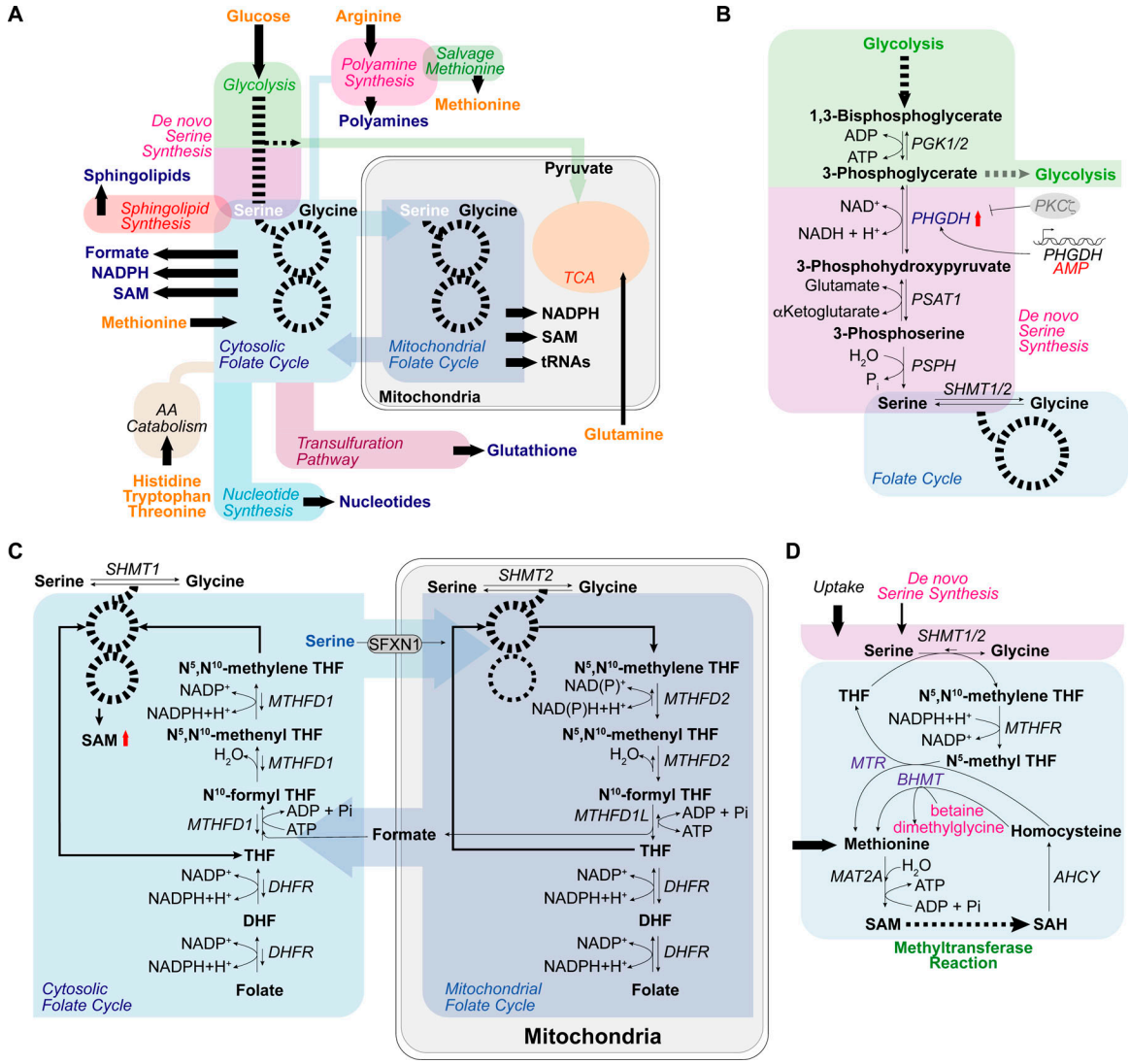

Figure 1. **Modular composition of the SGOCP. (A)** Scheme of the multimodular composition of the SGOCP, indicating its main inputs (orange) and outputs (blue) of the metabolic network. **(B)** Glycolysis and de novo serine synthesis modules coupled to the folate cycle. **(C)** Cytosolic and mitochondrial folate cycles, showing interconnected metabolites and main flux direction in cancer cells. The schematic also shows the transport of serine to the mitochondria through SFXN1. **(D)** One-carbon cycles showing the entry of one-carbon units from serine, the remethylation of homocysteine by methionine synthase and BHMT, and the generation of SAM.

and maintain cell homeostasis (Ducker and Rabinowitz, 2017; Locasale, 2013; Yang and Vousden, 2016; Fig. 1 A). Transcriptional analyses of different cancers have revealed that the SGOCP is generally not overexpressed in tumors (Hu et al., 2013; Mehrmohamadi et al., 2014). Rather, its distinct modules follow similar but not perfectly correlated cancer-dependent patterns (Mehrmohamadi et al., 2014). For example, while the nucleotide synthesis enzymes are consistently up-regulated in almost all tumors (Hu et al., 2013), other modules are more heterogeneous, showing a higher degree of intratumor and interpatient variability, as seen for the modules controlling the synthesis of serine, glutathione, betaine, cysteine, NADPH, pyruvate, taurine, and alanine, as well as for those controlling methylation (Mehrmohamadi et al., 2014). This indicates that while nucleotide synthesis is key for all tumor cells, other products of the SGOCP have context-dependent roles. Serine feeds into the central core of the SGOCP via serine hydroxymethyltransferase (SHMT), which converts serine (three carbons) into glycine (two carbons), transferring one carbon to tetrahydrofolate (THF; Fig. 1 B). Increasing folate species levels is, therefore, a mechanism by which to promote flux through the SGOCP. Although the up-regulation of the de novo serine synthesis module was initially reported to correlate with increased liver cell proliferation and tumorigenesis in rats (Snell, 1984; Snell et al., 1987; Snell and Weber, 1986), and despite the known protumorigenic effects of folate species in child leukemias, the importance of serine de novo synthesis in human tumors was not demonstrated until two decades later. Two studies simultaneously reported that the first, and rate-limiting, enzyme of de novo serine synthesis, phosphoglycerate dehydrogenase (PHGDH), is important in tumorigenesis (Locasale et al., 2011; Possemato et al., 2011). Possemato et al. (2011) identified PHGDH, and other

SGOCP enzymes, to be key points of vulnerability during breast cancer tumorigenesis, from an in vivo shRNA-based loss-of-function screen of metabolic genes of clinical significance in breast cancer. Locasale et al. (2011), using a combined approach, identified the de novo biosynthesis of serine and glycine as being a major metabolic route for the utilization of glucose-derived carbons in certain human cancer cell lines. Consistently, PHGDH was found to be amplified and/or overexpressed in human breast cancer and melanoma, indicating its potential clinical relevance (Locasale et al., 2011).

PHGDH was later shown to promote cell survival under low-glucose conditions in colorectal cancer (CRC); such conditions are often found in nutrient-deprived tumor microenvironments (TMEs; Ma et al., 2013; Fig. 1 B). PHGDH up-regulation in CRC occurs as part of a metabolic reprogramming that is orchestrated by the loss of the tumor suppressor PKCζ. Under homeostatic conditions, PKCζ represses PHGDH expression and inhibits its catalytic activity by direct phosphorylation (Ma et al., 2013), thereby reducing glucose-derived carbon flux through the SGOCP. Upon PKCζ loss, colon cancer cells can switch to glutamine for anaplerosis, relieving their dependence on glucose and preventing apoptosis (Ma et al., 2013). Importantly, PHGDH up-regulation has been observed in PKCζ-deficient mouse intestinal cells in vivo. PHGDH expression was found to negatively correlate with PKCζ expression in CRC samples from human patients, highlighting its relevance in human cancer (Ma et al., 2013; Fig. 1 B). High levels of PHGDH and SHMT2 were also found in a subgroup of lung cancer patients with a poor prognosis (Zhang et al., 2017). Additionally, lung cancer cell lines with high PHGDH levels showed increased synthesis of serine, nucleotides, and glutathione and displayed higher proliferative activity in vitro and in vivo (Zhang et al., 2017). The SGOCP activity also fuels the synthesis of precursors needed for the epigenetically regulated phenotypic conversion of tumor cells to therapy-resistant and more aggressive variants, including the differentiation to a highly lethal prostate cancer (PCa) subtype termed neuroendocrine prostate cancer (NEPC; Kottakis et al., 2016; Reina-Campos et al., 2019). Importantly, human NEPC samples showed increased expression of the SGOCP components, while PHGDH inhibition was able to reduce tumor growth and NEPC differentiation in vivo (Reina-Campos et al., 2019; Fig. 1 B). In summary, high PHGDH activity is a key feature of certain tumor types, including melanoma, breast, colon, NEPC, and lung cancer, in which PHGDH is required for tumor cell proliferation and survival. In PCa, PHGDH also controls the phenotypic plasticity that allows tumor cells to develop drug resistance. These recent data support the notion that PHGDH is a major potential target for new cancer therapy. However, while some studies have developed small drug inhibitors with preclinical efficacy (Mullarky et al., 2016; Pacold et al., 2016; Wang et al., 2017), the importance of PHGDH as a therapeutic target in the clinic still needs to be proven. Additionally, the potential role of exogenous serine, and the secondary effects of targeting PHGDH, should be carefully considered when assessing the efficacy of these compounds in vivo (de Koning et al., 2004; Sullivan et al., 2019; Vandekeere et al., 2018; Yoshida et al., 2004).

Other enzymes of the SGOCP play a key role in tumorigenesis. Methylenetetrahydrofolate dehydrogenase 2 (MTHFD2) is consistently up-regulated in many cancer types, and its expression significantly correlates with poor clinical outcome in breast cancer, pancreatic carcinomas, renal cell carcinoma, and leukemia and in a particularly aggressive metabolic subtype of hepatocellular carcinoma (HCC; Bidkhori et al., 2018; Lehtinen et al., 2013; Lin et al., 2018; Liu et al., 2014; Nilsson et al., 2014; Noguchi et al., 2018; Reina-Campos et al., 2019; Tedeschi et al., 2015). MTHFD2 is a dual-action enzyme (dehydrogenase and cyclohydrolase) that catalyzes the reversible conversion of 5,10-methylene-THF into 10-formyl-THF in the mitochondria, while MTHFD1, its cytosolic counterpart, catalyzes an extra reaction (synthetase) to convert 10-formyl-THF into THF and formate (Fig. 1 C). In the mitochondria, synthetase activity is performed by a different enzyme called MTHFD1L, which connects the mitochondrial and cytosolic folate pools through the generation of formate and maintains central energy metabolism (Bryant et al., 2018; Momb et al., 2013; Pike et al., 2010; Fig. 1 C). MTHFD2 activity is key to the mitochondrial generation of folate products from the loaded THF obtained from the catabolism of serine by SHMT2 reactions, which are used for nucleotide synthesis to mediate the pro-anabolic functions of mTORC1 (Ben-Sahra et al., 2016; Villa et al., 2019). MTHFD2 promotes stemness, epithelial-mesenchymal transition, and therapy resistance (Lin et al., 2018; Nishimura et al., 2019; Fig. 1 C). MTHFD1L activity is important for embryogenesis and neural tube closure (Momb et al., 2013; Parle-McDermott et al., 2009) and seems to confer a metabolic advantage in HCC. But the extent to which this reflects a coordinated action with MTHFD2 is not yet known (Bidkhori et al., 2018; Lee et al., 2017). An intriguing aspect of MTHFD2 is its ability to use both NAD^+ and $NADP^+$ as cofactors to generate mitochondrial NADH and NADPH, respectively, while MTHFD1 can only use $NADP^+$ (Shin et al., 2017b; Fig. 1 C). However, the functional relevance of this dual specificity remains unknown.

The enzyme betaine-homocysteine methyltransferase (BHMT) reconstitutes homocysteine to methionine by accepting one-carbon units from betaine (trimethylglycine) in the presence of vitamin B12 in a zinc-dependent reaction (Fig. 1 D). Its deficiency in mice promotes fatty liver, HCC, and the accumulation of homocysteine and increases *S*-adenosylhomocysteine (SAH)/*S*-adenosylmethionine (SAM) ratios in most tissues (Teng et al., 2011). It also results in the depletion of polyamines and sphingolipids in certain tissues and a striking accumulation of liver triacylglycerols due to a defect in the synthesis of very-low-density lipoproteins. This defect in very-low-density lipoprotein generation is most likely the underlying cause of fatty liver and HCC in this knockout (KO) model (Teng et al., 2011). However, because this was a total-body KO, the increased incidence of HCC could also be due to other impaired non–cell-autonomous mechanisms (Teng et al., 2011). Future studies should address this important question with cell-specific conditional KO mouse models. Nevertheless, the BHMT KO phenotype recapitulates, to some extent, the potent oncogenic potential of metabolic deficiencies driven by the dietary restriction of choline and methionine, which have been used for

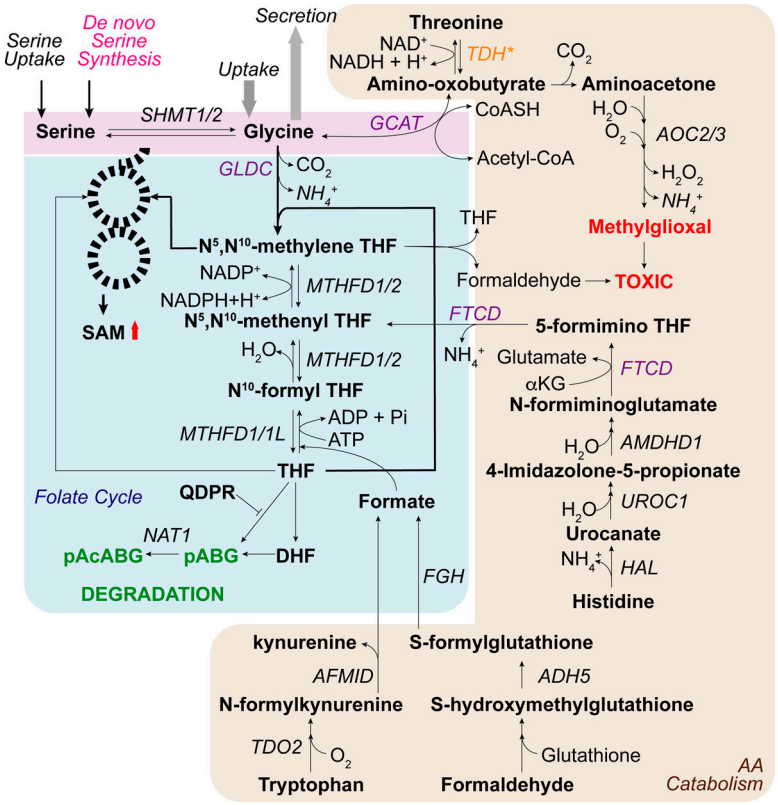

Figure 2. **Sources of one-carbon units.** Routes of entry of one-carbon units from the catabolism of amino acids, from metabolic pathways that detoxify secondary toxic byproducts and from those that prevent degradation of folate species. Toxic byproducts are colored in red. Degraded folate species are colored in green. The asterisk and orange color denote mouse enzyme with function not conserved in humans.

decades to create experimental models of cirrhosis, fatty liver, and HCC (Ghoshal et al., 1983; Newell et al., 2008). Thus, caution should be exerted when considering dietary deprivation as a potential therapeutic strategy for those amino acids that feed into these same reactions (Gao et al., 2019; Maddocks et al., 2017).

Another important aspect of the SGOCP is its role in the control of stem cell function, which is of relevance because the expression of adult stem-like features is closely associated with cancers of a poor prognosis (Smith et al., 2018). The metabolic state of mouse embryonic stem cells (ESCs) has been linked to the use of threonine as a one-carbon donor through the up-regulation of the enzyme threonine dehydrogenase (TDH; Wang et al., 2009). TDH activity generates glycine and acetyl-CoA to supply the SGOCP and the TCA, respectively (Wang et al., 2009). In turn, glycine contributes to SAM production, which sustains a histone methylation code that maintains stem cell pluripotency (Shyh-Chang et al., 2013; Fig. 2). However, human TDH lacks catalytic activity due to a genetic insertion of a premature codon (Edgar, 2002; Fig. 2). Thus, the relevance of threonine catabolism in supplying one-carbon units in humans is unclear. Conversely, high levels of α-ketoglutarate, generated by enhanced activity of phosphoserine aminotransferase 1 (PSAT1), can maintain the stemness of mouse ESCs by linking the SGOCP activity to a pluripotent epigenetic landscape (Hwang et al., 2016). Human ESCs require large amounts of methionine and overexpress several pathways of the SGOCP's methionine and folate cycle modules (Shiraki et al., 2014). In the context of cancer, tumor-initiating cells (TICs), which recapitulate key features of ESCs, have a similar dependence on methionine to maintain elevated levels of SAM synthesis (Wang et al., 2019). Indeed, basal and stem cell genetic programs are activated during the development of acquired resistance through mechanisms of cellular plasticity (Davies et al., 2018; Smith et al., 2015). Drug resistance also occurs through increased SGOCP activity in PCa, melanoma, pancreatic carcinomas, and non–small cell lung carcinoma (NSCLC; Reina-Campos et al., 2019; Ross et al., 2017). The less proliferative nature of TICs might underlie their different utilization of key aspects of the SGOCP, likely by shifting the pathway output from nucleotide generation to that of a reductive environment and the synthesis of SAM. However, how tumor cells simultaneously support stem cell features and highly proliferative traits, such as those found in NEPC, remains largely unexplored. We also know little about the mechanisms that cells use to switch from a TIC-like state to a highly proliferative tumor cell. Despite these remaining questions, together, these observations reveal a fundamental role for the SGOCP in tumorigenesis.

Sources of one-carbon units

A key question regarding the SGOCP activation is the source of the one-carbons. Serine is the default and major donor of one-carbon units (Yang and Vousden, 2016). Alternatively, glycine can also donate one-carbons through the glycine decarboxylase complex (GLDC) and replace the need for serine under some circumstances (Zhang et al., 2012; Fig. 2). The study by Zhang et al. (2012) showed that GLDC overexpression can increase pyrimidine glycolytic activity and synthesis to sustain cancer cell proliferation. This route directly supplies the 5,10-methylene-THF pool, bypassing the requirement for serine and even

contributing to the de novo generation of this metabolite (Fig. 2). The authors also showed that several SGOCP enzymes, including GLDC, are up-regulated in NSCLC TICs, and GLDC overexpression alone, but not that of its enzymatically inactive versions, is sufficient to induce transformation in 3T3 cells. Moreover, potent oncogenes, such as MYC, PI3K, and KRAS, were reported to promote GLDC expression, while its inhibition impaired in vitro growth of TICs derived from human NSCLC. These findings demonstrated the transforming capacity of increased SGOCP flux and the ability of glycine to replace serine as a one-carbon donor in vivo. The overexpression of PSAT1, phosphoserine phosphatase, or SHMT2 alone had transformation capacity in 3T3 cells, while the cytosolic version of SHMT (SHMT1) had no transforming activity. This led to the conclusion that the mitochondria-specific branch of the SGOCP is the one important for cellular transformation (Zhang et al., 2012).

A key question arising from these studies relates to the relative importance of glycine versus serine for oncogene function in TICs and in non-TIC tumor cells. Other in vitro human cellular models have shown that in the absence of serine, glycine cannot sustain cancer cell proliferation (Labuschagne et al., 2014; Fig. 2). In TICs, glycine usage through GLDC activity is favored, although it is not needed for growth in several other human cancer cell lines in 2D culture (Labuschagne et al., 2014). Notably, in tumor cell lines cultured in vitro, the glycine generated by serine catabolism is excreted into the media instead of being used by GLDC (Labuschagne et al., 2014; Reina-Campos et al., 2019; Fig. 2). A potential explanation for this paradoxical waste of glycine is that highly proliferative non-TIC tumor cells prefer serine when available and use glycine only when serine is limited (Labuschagne et al., 2014). This might occur in tumors where two cell populations coexist: a larger population of highly proliferative cells that consume serine in large quantities and make glycine, and a smaller TIC population that uses glycine in place of competing for the scarcer serine. This hypothetical scenario would also benefit the tumor because it would lower the levels of interstitial glycine, which at high concentrations is reportedly detrimental to tumor cell growth for reasons that are not yet known (Labuschagne et al., 2014; Fig. 2). One possible explanation is that high glycine levels limit the number of one-carbon units for nucleotide synthesis, since a glycine overflow can deplete the 5,10-methylene-THF pool by forcing the reversed SHMT reaction (Labuschagne et al., 2014). Another possible explanation is that glycine decarboxylation generates CO_2 and ammonia, which can be toxic if not properly disposed of (Kikuchi et al., 2008). Intriguingly, cells fed only glycine can maintain glutathione synthesis, but not the nucleotide synthesis rate, even though glycine alone can theoretically generate all the nucleotide precursors (Labuschagne et al., 2014). In gliomas, which have high levels of GLDC and SHMT2, glycine accumulation caused by high SHMT2 activity cannot be cleared by decarboxylation if GLDC is inhibited, resulting in the production of the toxic byproducts aminoacetone and methylglyoxal. The subsequent toxicity cannot be rescued by the addition of formate in a human adherent glioblastoma multiforme cell line (Kim et al., 2015; Fig. 2).

Other amino acids can also fuel the SGOCP. Histidine catabolism can replenish the cytosolic pools of 5,10-methenyl-THF, through the rate-limiting actions of formimidoyltransferase cyclodeaminase (FTCD; Kanarek et al., 2018; Fig. 2). This pathway becomes relevant in the context of methotrexate therapy. Methotrexate is a potent inhibitor of dihydrofolate reductase (DHFR) that depletes the THF pools and blocks cell proliferation (Goodsell, 1999; Rajagopalan et al., 2002). Thus, FTCD inhibition sensitizes tumor cells to methotrexate treatment by blocking alternative sources to replenish the folate cycle. Thus, while not a major supply route, histidine catabolism can play a crucial role when the main pathways become blocked. One-carbon units might also arise from the detoxification of secondary byproducts generated in core reactions of the SGOCP. Thus, certain oxidant-prone folate species, such as DHF, THF, and 5,10-methylene-THF, can spontaneously generate formaldehyde from the methylene link that connects pteridine with p-aminobenzoylglutamic acid (Brewer and Chang, 2015; Chippel and Scrimgeour, 1970; De Brouwer et al., 2007; Fig. 2). In this way, the released formaldehyde, which accumulates in blood in the range of 20–100 µM, acts as a carcinogen that cross-links DNA and proteins and triggers DNA damage (Burgos-Barragan et al., 2017). Importantly, certain enzymes, such as ALDH5A1, DNA repair proteins, and antioxidant vitamins such as ascorbate, offer a combined protective mechanism against THF-derived formaldehyde (Burgos-Barragan et al., 2017). In this mechanism, glutathione spontaneously reacts with formaldehyde to be posteriorly converted to formate through the sequential actions of ALDH5 and *S*-formyl glutathione hydrolase. This generates a recycling pathway that contributes significantly to one-carbon units for the synthesis of purine and pyrimidine intermediates, for de novo ATP synthesis, and up to ∼20% of plasma formate levels (Burgos-Barragan et al., 2017).

Thus, although the main one-carbon source for the SGOCP is serine, other sources exist, including the catabolism of glycine, histidine, tryptophan, or threonine, as well as the detoxification of formaldehyde. Diet-derived one-carbons from folate species and vitamins, such as betaine, vitamin B12, and choline, might also be an important exogenous supply of carbon and enzymatic cofactors for the SGOCP (Fig. 2).

Limiting the inputs exposes key outputs

Why does PHGDH deficiency impair tumor cell growth in the presence of exogenous serine? One possibility is that de novo generated serine is particularly important for nucleotide synthesis (Pacold et al., 2016). However, the amount of serine synthesized in cells, although significant in some contexts, is small compared with extracellular uptake in nutrient-replete conditions (Davis et al., 2004; Furuya, 2008; Gregory et al., 2000; Kalhan and Hanson, 2012). Alternatively, PHGDH activity might be needed by tumor cells to maintain other processes, beyond de novo serine synthesis. Consistent with this possibility, PHGDH inhibition affects nucleotide synthesis independently of serine utilization (Reid et al., 2018). This might represent a potential mechanism by which PHGDH maintains the balance between the pentose phosphate pathway (PPP) and the biosynthetic activities of the TCA (Reid et al., 2018). By

diverting glucose-derived carbons to de novo serine synthesis, PHGDH might thus actively maintain the anabolic reactions that feed into the central carbon metabolism; for example, the transamination reactions by PSAT1, downstream of PHGDH, that contribute to anabolism and that can partly sustain TCA activity (Possemato et al., 2011). PHGDH can also produce significant amounts of the oncometabolite 2-hydroxyglurate from α-ketoglutarate (Fan et al., 2015), which could further promote tumorigenesis (Losman et al., 2013), although the relevance of this atypical 2-hydroxyglurate source, compared with that from isocitrate dehydrogenase (IDH), remains to be clarified.

The potential contribution of the SGOCP to tumor cell growth can also be determined by the relative contribution of PHGDH activity to the TME and by the availability of serine in this environment. Increased de novo serine biosynthesis might be a strategy tumors use to thrive in conditions of low serine availability (Sullivan et al., 2019). However, while there is certainly tumor variability (Kamphorst et al., 2015), serine concentrations in the TME are reportedly high overall (Goveia et al., 2016), which calls into question whether the generation of more serine would be advantageous to cancer cells in a high-serine environment. Evidence also suggests that not all tumors are sensitive to the dietary restriction of serine (Pacold et al., 2016; Possemato et al., 2011). Thus, it remains unclear whether extracellular serine and PHGDH are the only players that regulate the SGOCP in cancer. Perhaps, instead, an overall decrease or shift in the requirement of the SGOCP outputs, non–cell-autonomous effects, or an increase in its uptake capacity can modify a tumor's sensitivity to extracellular serine. In fact, only nucleotides appear to be universally required by tumor cells to grow (Vander Heiden et al., 2009), while other products of the SGOCP pathway might depend on tumor type and context. We next discuss the specific generation of the main products of the SGOCP metabolism and their physiological relevance, with the goal of better understanding how context determines the role of the SGOCP activity in tumorigenesis.

SAM. All methyltransferase reactions in mammalian cells rely exclusively on the methyl donor SAM, an important SGOCP output (Maddocks et al., 2016). SAM is generated by methionine adenosyltransferase 1A (cytosol) or 2A (in the mitochondria) from the transfer of adenosine from an ATP molecule to methionine (Fig. 3 A). SAM synthesis lies at the SGOCP core, and its abundance is tightly regulated; moreover, its physiological concentration is limiting for the activity of histone and DNA methyltransferases (Reid et al., 2017). Thus, levels of SAM, and of its derivative SAH, can directly influence the epigenetic landscape of tumor cells by modulating the activity of key epigenetic enzymes, to ultimately dictate their cell fate (Caudill et al., 2001; Cuyàs et al., 2018; Kottakis et al., 2016; Kraus et al., 2014; Mentch and Locasale, 2016; Mentch et al., 2015; Reina-Campos et al., 2019; Ulanovskaya et al., 2013; Fig. 3 A).

In PCa, increased SAM production via an mTORC1-mediated up-regulation of the activating transcription factor 4 (ATF4)/SGOCP axis by PKCλ/ι deficiency was found to contribute to increased cell lineage plasticity and to the acquisition of resistance to targeted therapy in human cancer cell lines and in vivo mouse models (Reina-Campos et al., 2019). In this context, the inhibition of PHGDH or of the DNA methyltransferase activity was able to reestablish normal SAM pools and to rescue cell differentiation and proliferation in vitro and in vivo (Reina-Campos et al., 2019). PKCλ/ι-deficient cells also showed increased incorporation of methionine-derived carbons into 5-methyl-cytosine, which depended on extracellular serine (Reina-Campos et al., 2019). This suggests that while the de novo synthesis of serine was increased by PKCλ/ι deficiency, it was likely not enough to prevent serine starvation from affecting SAM pools (Reina-Campos et al., 2019; Fig. 3 A). In another system, LKB1 deficiency in the context of mutant KRAS increased SAM generation, with DNA methylation occurring on intergenic and repetitive elements in human cancer cell lines. The consequent silencing of retrotransposon elements was proposed to promote tumorigenesis, but without significantly affecting gene expression (Kottakis et al., 2016). A similar effect was reported during metformin-induced AMPK activation in breast epithelial cells (Cuyàs et al., 2018).

Interestingly, increased SAM production can also be handled by diverting SAM to "methyl sinks," metabolite pools that offer a stable product, such as 1-methyl nicotinamide, in which to store methyl units. 1-Methyl nicotinamide is partly generated by nicotinamide *N*-methyltransferase (Ulanovskaya et al., 2013), increased activity of which is seen in some cancer types and induces histone hypomethylation by depleting the available pool of SAM for histone methyltransferases (Ulanovskaya et al., 2013). Another proposed methyl sink is the lipid phosphatidylcholine, produced by the methylation of phosphatidylethanolamine by phosphatidylethanolamine *N*-methyltransferase (Ulanovskaya et al., 2013; Ye et al., 2017). The storage of SAM involves a methyltransferase reaction; the respective increase in SAH contributes to the synthesis of glutathione through homocysteine (transulfuration pathway) to increase the redox power of the cell (Ye et al., 2017). Other methyl sinks include histone tails, such as K36, K79, and K4 (Ye et al., 2017; Fig. 3 A). How the epigenetic landscape harnesses increased levels of SAM and the storage of methyl groups to fulfill specific epigenetic programs remains to be fully defined. We also do not know the extent to which the different mitochondrial and cytosolic SAM pools affect methylation reactions in the nucleus, nor whether an independent source of nuclear SAM exists.

These questions are very important because SAM accumulation can have a much broader metabolic impact when it occurs as a consequence of decreased methyltransferase activity. For example, loss of glycine *N*-methyltransferase in the liver promotes SAM accumulation, which is associated with hepatomegaly and precedes HCC. While the causality has not yet been fully established, SAM accumulation in this context correlates with increased lipogenesis, polyamine biosynthesis, and transulfuration activity (Hughey et al., 2018). All of these reactions might account for the increased anabolic activity required for tumorigenesis.

Formate. Cancer cells in culture secrete formate, perhaps because they run excess carbons through the SGOCP in an apparent waste of biomass (Meiser et al., 2016). In mice, the release of formate is a hallmark of oxidative spontaneous intestinal adenomas and mammary tumors (Meiser et al., 2018).

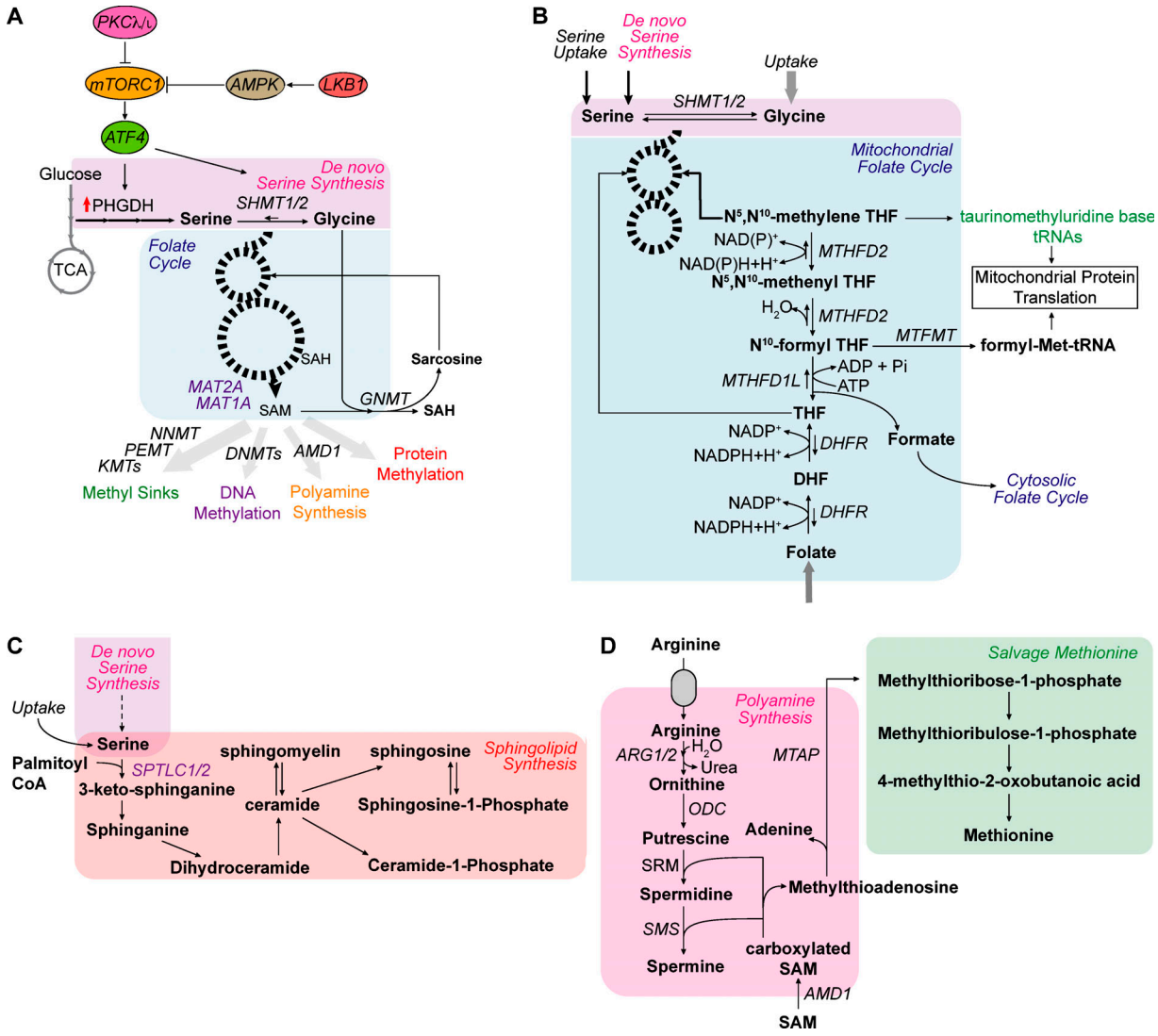

Figure 3. **Products of the SGOCP. (A)** Metabolic reactions and upstream regulators that control SAM synthesis. **(B)** The mitochondrial folate cycle module that contributes to the generation of mature tRNAs for the translation of mitochondrial-coded proteins. **(C)** Reactions that control sphingolipid synthesis. **(D)** Polyamine synthesis coupled to the salvage pathway of methionine.

Interestingly, a blockade of formate production impairs tumor growth in vivo (Meiser et al., 2018), possibly because generating formate from 5,10-methylene-THF produces 1 molecule of ATP, or 3.5 molecules of ATP if the NADH produced by MTHFD2 is coupled to oxidative phosphorylation (Meiser et al., 2016). Thus, this apparent waste of biomass could actually constitute a quick way to boost energy production, reminiscent of the Warburg effect. However, formate release might also contribute to tumor growth in vivo in a non–cell-autonomous manner. For example, its release could have an impact on the metabolism of the tryptophan-derived metabolite kynurenine and, in turn, modulate the T cell–mediated antitumor response, as previously suggested (Cervenka et al., 2017; Fig. 2).

Nucleotides. Tumor cells increase their demand for nucleotides to sustain high proliferation rates. The SGOCP produces the nucleotide precursors glycine and N^{10}-formyl-THF, which contribute to purine ring formation (Ben-Sahra et al., 2016; Villa et al., 2019). Glycine can also contribute to pyrimidine synthesis via the generation of 5,10-methylene-THF and deoxythymidine monophosphate via GLDC and thymidylate synthetase, respectively (Zhang et al., 2012). Thus, the SGOCP provides several components in the synthesis of pyrimidines and purine precursors and plays a key role in sustaining cancer cell growth (Lane and Fan, 2015; Villa et al., 2019).

tRNAs. Folate pools generated in mitochondria provide vital support for mitochondrial-coded protein translation via the methylation and formylation of mitochondrial tRNA pools (Minton et al., 2018; Morscher et al., 2018; Tucker et al., 2011). SHMT2 deficiency or MTFMT mutations impair the formylation of the initiating methionine tRNA (formyl-Met-tRNA), affecting the translation of mitochondrial-coded proteins, such as COX1, and simultaneously reducing oxidative phosphorylation in human cell lines (Minton et al., 2018; Tucker et al., 2011). Additionally, SHMT2-generated 5,10-methylene-THF reportedly

contributes to the formation of the taurinomethyluridine base of other specific tRNAs, such as lysine and leucine (Morscher et al., 2018). Thus, tRNA modification by different one-carbon pools in the mitochondria is required for adequate protein translation of oxidative phosphorylation complexes and is probably the cause of specific several inborn errors of mitochondrial metabolism. This also explains the impaired basal respiration rate observed in folate deficiency that can cause neural tube defects during embryogenesis (Fox and Stover, 2008; Morscher et al., 2018; Pendleton, 1969; Fig. 3 B).

Redox power. The SGOCP is an important producer of reducing power in the form of NADPH and significantly complements the main source coming from glucose oxidation via the PPP (Fan et al., 2014). The SGOCP-dependent NADPH production is led by the coordinated actions of MTHFR and MTHFD enzymes through the catabolism of serine but not of glycine, mostly by the mitochondrial branch of the pathway (Chen et al., 2019; Ducker et al., 2016). At the same time, the SGOCP relies on cytosolic NAPDH supplied by the activity of the PPP (Chen et al., 2019). While several sources of NADPH exist, inhibition of the PPP by deletion of glucose-6-phosphate dehydrogenase raises NADP and impairs folate-mediated biosynthesis via inhibition of DHFR in CRC cell lines, suggesting that PPP plays a key role in maintaining NADP/NADPH ratios (Chen et al., 2019). While ME1 and IDH1 are also producers of cytosolic NADPH in this setting, their contribution might be context dependent (Chen et al., 2019). For example, in gliomas, IDH1 is required for NADPH production (Calvert et al., 2017). Other findings revealed a key function of ME2/3 in generating NADPH (Dey et al., 2017). In sum, the SGOCP has a positive net contribution of NADPH (in the mitochondria) but is subject to the availability of NADPH in the cytoplasm.

Sphingolipids. As components of the lipid bilayer, sphingolipids are important for the cell's structural integrity, and as bioactive lipid messengers, for intracellular signal transduction (Ryland et al., 2011). Sphingolipid metabolites include ceramides, sphingosine, sphingomyelin, dihydroceramide, glycosylated ceramides, and sphingoid long-chain bases (Ryland et al., 2011). Sphingoid bases are the sphingolipid structural unit and are obtained from salvage routes or via de novo synthesis, which requires serine. Serine is combined with palmitoyl-CoA to generate 3-keto-sphinganine by serine palmitoyl transferase (SPT) in the first committed rate-limiting reaction of sphingolipid synthesis (Braun et al., 1970; Braun and Snell, 1968; Merrill, 2011; Stoffel et al., 1967). Further enzymatic processing converts sphingoid bases into more complex lipids. Ceramide synthesis also relies on serine (Gao et al., 2018). Indeed, limited serine availability in tumor cell lines can impair ceramide synthesis and mitochondrial function (Gao et al., 2018), and in these conditions, SPT metabolizes alanine and glycine, generating sphingoid bases that lack the hydroxyl group (deoxysphinganine and deoxymethylsphinganine) and are toxic to the cell (Esaki et al., 2015; Sayano et al., 2016; Fig. 3 C). However, the physiological and functional relevance of promiscuous SPT activity in cancer has yet to be explored. Paradoxically, ceramide levels increase in response to most chemotherapeutic agents and possibly contribute to cell death (Ryland et al., 2011). In fact, some mechanisms of resistance to chemotherapy could be due to defects in ceramide synthesis (Wang et al., 1999). Thus, it seems that ceramide levels below or above a certain threshold can trigger cytotoxic responses through independent mechanisms and represent important functional outputs of the SGOCP. Sphingolipid enzyme inhibitors and sphingolipid mimetics have been tested as antitumor agents, although never in combination with other inhibitors of the SGOCP (Ogretmen and Hannun, 2004; Ryland et al., 2011).

Polyamines. Polyamines are derivatives of arginine catabolism generated by the sequential actions of arginase and ornithine decarboxylase to produce putrescine (Casero et al., 2018). While ornithine decarboxylase is the first rate-limiting step in putrescine synthesis, the generation of higher polyamines needs the synthesis of decarboxylated SAM, which is sequentially incorporated into putrescine to generate spermidine and spermine, respectively (Casero et al., 2018). Thus, SAM decarboxylase (AMD1), which is the second rate-limiting step, has to compete with hundreds of other SAM-consuming enzymes. Nevertheless, polyamines accumulate in rapidly growing tissues, including cancers, to levels that are detectable even in excreted urine (Bachrach, 2004; Caldarera et al., 1965; Raina and Jänne, 1968; Russell et al., 1971). The aminopropylation reaction that incorporates decarboxylated SAM into putrescine and spermidine generates methylthioadenosine, which is rapidly recycled to the methionine salvage pathway by methylthioadenosine phosphorylase, generating adenine and methylribose-1-phosphate to reconstitute methionine (Fig. 3 D). These are important observations, but a number of key questions remain to be addressed. Why is polyamine biosynthesis up-regulated in cancer? How does it contribute to tumorigenesis? And how does it fit with the other roles of the SGOCP? Almost certainly, the methionine salvage pathway is not the reason for increased polyamine synthesis. This is because methylthioadenosine phosphorylase is usually deleted or suppressed by DNA methylation and is possibly a tumor suppressor (Kadariya et al., 2009). However, one key function of polyamine synthesis is to supply derivatives that are used as substrates for posttranslational modification, such as hypusine, which posttranslationally regulates eIF5A to prevent ribosomal stalling during mRNA translation (Shin et al., 2017a). Decarboxylated SAM, generated by AMD1, might also constitute a potential cancer vulnerability. In fact, AMD1 inhibitors have been developed and show potent antitumor activity in vitro, but with severe toxicities in vivo (Gamble et al., 2012; Regenass et al., 1992; Fig. 3 D). In sum, polyamines are highly functional bioactive metabolites that are tightly linked to the SGOCP activities and are often overlooked when considering the metabolic reach of this pathway.

The SGOCP regulation

Four main regulatory tiers control the SGOCP's metabolic activity: (1) cellular signaling through nutrient sensors, which couples the cells' energetic and nutrient status to the SGOCP activity; (2) the transcriptional and epigenetic regulation of the SGOCP genes, which determines overall coordination of the SGOCP modules; (3) pathway module subcellular compartmentalization, which ensures that enzymatic reactions occur in

favorable conditions, increases redundancy, and provides additional regulation over pathway inputs and outputs; and (4) posttranslational modification of enzymes and metabolites, together with feedback loops, enzymatic complex formation, and cofactor availability, which also regulate the catalysis of key steps of this metabolic network. Together, these mechanisms provide robust control across the SGOCP, and many are deregulated in human tumors, as we discuss in this section.

Nutrient and energy sensing are coupled to the SGOCP transcriptional and epigenetic regulation

Cells have mechanisms to increase serine and glycine biosynthesis when their availability decreases to promote cell survival (Ye et al., 2012). For example, p53 is activated upon serine deficiency, which induces a p21-dependent cell cycle arrest. This allows serine-depleted SGOCP metabolites to be diverted for glutathione synthesis to combat oxidative stress, concomitant with the shutdown of aerobic glycolysis in favor of TCA activity to promote survival (Maddocks et al., 2013). The sensing of serine depletion occurs through an enzyme termed general control nonderepressible 2 (GCN2), which detects the ratio of unloaded to loaded tRNAs and triggers the translation of ATF4, through the unfolded protein response (UPR) pathway to promote the coordinated transcription of the SGOCP enzymes (Ye et al., 2010). ATF4 can also be triggered indirectly by the inhibition of intracellular pathways that contribute to the serine pool, such as lactate dehydrogenase (Pathria et al., 2018). Increased ATF4 up-regulates the SGOCP enzymes to drive de novo serine and glycine production and glutamine uptake and that of other nutrients (Pathria et al., 2018; Ye et al., 2010). ATF4 up-regulation also promotes asparagine synthesis by actively transcribing asparagine synthase, which is then released in exchange for arginine, serine, and histidine uptake to maintain mTORC1 activation (Krall et al., 2016). Additionally, lack of PKM2 allosteric activation by serine can trigger the accumulation of glycolytic intermediates of PKM2-expressing cells to sustain mTORC1 activation (Ye et al., 2012). NRF2, a potent inducer of ATF4 expression, redirects glucose and glutamine metabolism to pro-anabolic pathways that sustain cell proliferation and enhance redox defense (Mitsuishi et al., 2012). In fact, the up-regulated expression of NRF2, ATF4, and the SGOCP genes delineates a subset of NSCLC tumors that show the increased expression of serine and glycine synthesis enzymes and are associated with poor prognosis (DeNicola et al., 2015). These NSCLC cells can sustain nucleotide synthesis to remain highly proliferative (DeNicola et al., 2015). ATF4 is thus central to the control of mTORC1 activity in response to the loss of key nutrients by the up-regulation of the serine biosynthetic pathway (Fig. 4).

Interestingly, ATF4 was initially identified as being important to maintain mTORC1 activity and for glucose homeostasis (Adams, 2007; Seo et al., 2009) and is now considered to be a master regulator of the anabolic response downstream of mTORC1 activation that maintains the aminoacyl-tRNA pool (Adams, 2007). ATF4 also coordinates nucleotide synthesis and increases SAM levels (Ben-Sahra et al., 2016; Park et al., 2017; Reina-Campos et al., 2019). Interestingly, increased SAM can be directly sensed by BMT2 (SAMTOR) to feedback-activate mTORC1 by relieving the inhibitory effect of Rag A/B GTPase NPRL2 (GATOR1; Gu et al., 2017). Thus, SAM-dependent mTORC1 activation is one mechanism by which mTORC1 can sense the levels of the SGOCP activity (Gu et al., 2017; Fig. 4). However, the in vivo relevance of this positive regulatory loop in cancer remains unexplored.

ATF4 is translationally induced upon activation of the PERK signaling arm of the UPR (Harding et al., 2000), which ensures correct protein synthesis in the ER and triggers an antistress response when activated (Walter and Ron, 2011). Thus, UPR-mediated ATF4 activation is intended to counteract proteotoxic stress by promoting prosurvival genes. However, chronic and sustained UPR signaling can bring ATF4 expression over a threshold, causing it to promote apoptosis by inducing proapoptotic genes (Qing et al., 2012), as occurs during chronic glutamine deprivation in neuroblastoma cells (Qing et al., 2012). It is paradoxical that ATF4 induction occurs through the sensing of nutrient-specific stressors, upon pro-anabolic signaling triggered by nutrient abundance, or through the metabolic reprogramming that favors tumorigenesis. However, how ATF4 differentially modulates its transcriptional activity under stress-induced or pro-anabolic signals is not well understood.

Several other transcription factors also modulate the SGOCP. PGC1α/ERRα directly represses several SGOCP enzymes downstream of the metformin-dependent activation of AMPK, which underlies the sensitivity of ERRα-positive (ER^+) breast cancer to the DHFR antagonist methotrexate (Audet-Walsh et al., 2016). Conversely, estrogens promote an ERα-dependent increase in polyamine and purine synthesis via metabolic reprogramming of the mitochondrial branch of the SGOCP (Zhu et al., 2018). Also in ER^+ breast cancer, targeting of ESRP1 down-regulates the lipogenic program and PHGDH expression and stops growth of endocrine-resistant breast cancer cells (Gökmen-Polar et al., 2019; Fig. 4).

SREBP1 controls lipogenic biosynthesis genes and the expression of the SGOCP enzymes that produce SAM (Walker et al., 2011; Fig. 4). Interestingly, the maturation of fully functional nuclear SREBP1 depends on phosphatidylcholine levels, and phosphatidylcholine is generated by the SAM-dependent methylation of PE. Thus, SAM levels can modulate SREBP1 function to control a lipogenic program and alter the transcription of several SGOCP enzymes. In turn, SREBP1 can feed back to control the expression of SAM biosynthetic enzymes (Walker et al., 2011). While not a canonical transcription factor, chromatin-bound Mdm2 can also regulate the SGOCP genes to control redox cellular status and amino acid metabolism independently of p53 (Riscal et al., 2016).

Epigenetic regulation also controls the SGOCP. Such regulation poises tumor cells to rapidly up-regulate most of the SGOCP reactions and to increase the pathway's basal activity. This is achieved partly by the histone methyltransferase G9A (Ding et al., 2013). In addition, the histone demethylase KDM4C removes the repressive histone mark H3K9me3 and activates genes involved in amino acid biosynthesis and transport, including ATF4 (Zhao et al., 2016; Fig. 4).

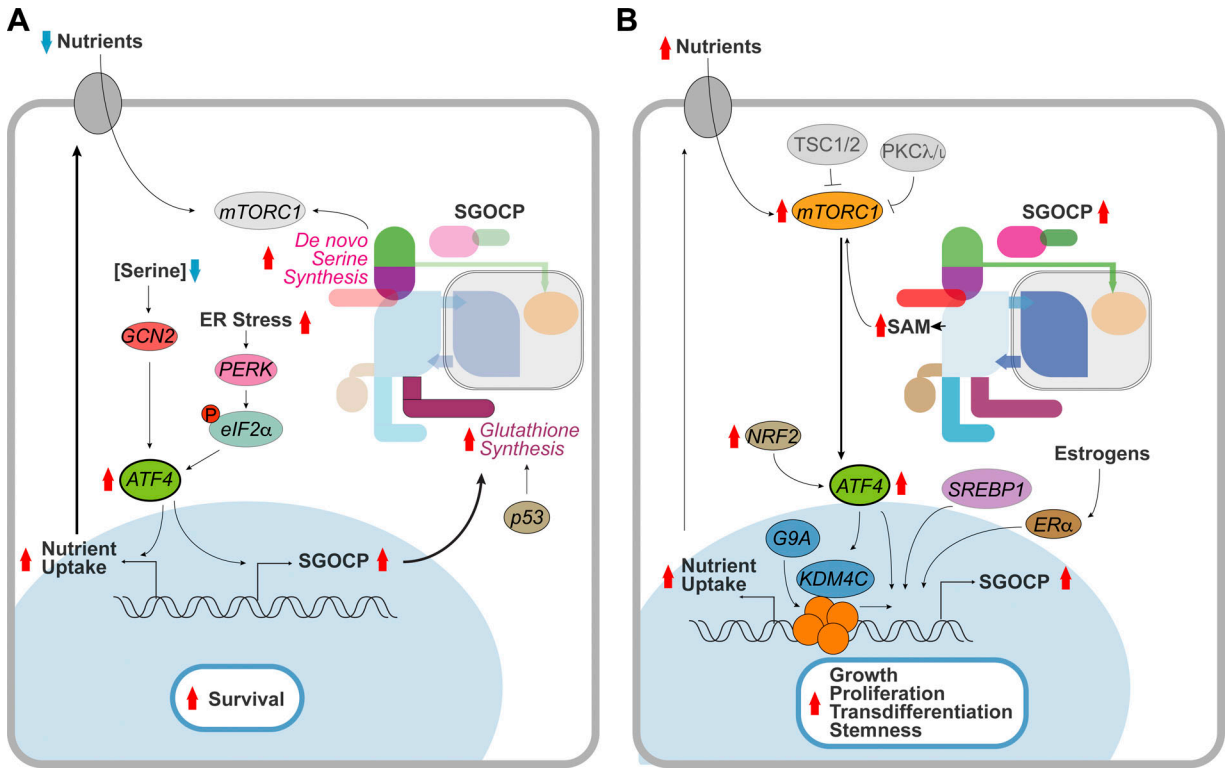

Figure 4. **Regulation of the SGOCP. (A and B)** Schematic showing the SGOCP regulation in nutrient-poor (A) and nutrient-rich (B) conditions. **(A)** Nutrient or ER stress activates the prosurvival functions of ATF4 by increasing nutrient uptake and promoting the machinery of intracellular amino acid biosynthesis to maintain mTORC1 activity. p53 is also activated to promote glutathione synthesis which, together with ATF4, increases cell survival. **(B)** In nutrient-rich conditions, the hyperactivation of mTORC1 and/or NRF2 activation leads to an increase in the SGOCP activity through ATF4. SREBP1 and estrogen-dependent ERα activation also up-regulate the SGOCP. mTORC1 is activated via the sensing of SAM levels. The anabolic activation of ATF4 leads to an increase in cell growth and proliferation and to transdifferentiation and stemness.

Compartmentalization, enzyme complex formation, and posttranslational regulation

A key mechanism that helps to generate metabolic products based on demand is the subcellular compartmentalization of the SGOCP reactions. Although the SGOCP has both cytosolic and mitochondrial compartments, cancer cells most heavily rely on the mitochondrial branch. Consequently, many mitochondrial, but not cytosolic, SGOCP enzymes are up-regulated in cancer (Hu et al., 2013). This also shapes which enzymatic alterations can contribute to cancer. For example, SHMT2 (mitochondrial), but not SHMT1 (cytosolic), has a transforming effect when overexpressed in 3T3 fibroblasts (Zhang et al., 2012).

Many of the SGOCP's enzymes also rely on the NADPH/NADP ratio, with the mitochondrial matrix having a much higher NADP/NADPH ratio than that of the cytosol (Tibbetts and Appling, 2010). Cells use this by uncoupling the SGOCP mitochondrial branch from the cytosolic one, using the oxidant-prone mitochondrial environment to generate formate from 5,10-methylene-THF (Ducker et al., 2016; Tibbetts and Appling, 2010; Fig. 1 C). Mitochondrial formate is shuttled to the cytosol, where it is converted by MTHFD1 into N10-formyl-THF, which is then diverted for nucleotide production. Because formate passively diffuses in and out of the mitochondria, it is still not known whether cells maintain separate formate pools in the mitochondria and cytosol. When the mitochondrial pathway is abolished in cancer cells, they become dependent on extracellular serine to make one-carbon units, and on glycine to produce glutathione, suggesting that the cytosolic SGOCP cannot sustain both de novo serine and glutathione synthesis (Ducker et al., 2016). Mitochondrial-produced formate also helps to keep cytosolic THF pools low by promoting its conversion to N10-formyl-THF. When mitochondrial formate production is blocked, it induces the cytosolic oxidation of THF, which can overwhelm the repair actions of quinoid dihydropteridine reductase (Zheng et al., 2018). Thus, compartmentalization helps to prevent THF degradation and a net flux of formate to cytosolic N10-formyl production, thereby minimizing the production of cytotoxic byproducts while maintaining a high SGOCP activity (Burgos-Barragan et al., 2017; Zheng et al., 2018; Figs. 1 C and 2).

For compartmentalization to occur, serine and glycine have to be actively transported into the mitochondria by sideroflexin 1 (SFXN1)/SFXN3 and SLC25A38, respectively (Kory et al., 2018). Deletion of SFXN1, and its close homologue SFXN3, made several cell lines auxotrophic for glycine and impaired mitochondrial function, possibly by affecting mitochondrial-coded proteins (Kory et al., 2018), as previously suggested (Fig. 1 C). The manipulation of serine transport via the modulation of SFXNs offers a promising approach for studying the functional relevance of the SGOCP's mitochondrial branch in cancer. However, their

specificity for other amino acids, and their dependence on and compensation by other homologues, still need to be clarified.

Posttranslational modifications are also important for the regulation of the SGOCP. For example, PHGDH activity is inhibited by PKCζ phosphorylation and its protein stability is controlled by the deubiquitinating enzyme, JOSD2 (Ma et al., 2013; Zhang et al., 2017). Additionally, the PHGDH oligomerization state dictates its catalytic rate (Mullarky et al., 2016) and changes in its tertiary structure modify its activity and ability to sustain cancer cell proliferation (Mattaini et al., 2015; Mullarky et al., 2016).

Together, these studies highlight that several regulatory mechanisms fine-tune the specific modules of the SGOCP to increase the generation of specific products and the regulation of the pathway's overall activity. These mechanisms could potentially be exploited therapeutically.

The SGOCP in nontumor cells of the TME

The SGOCP's metabolic activity is also important for the many nontransformed cell types in the TME. Naive T cell activation induces a pro-anabolic reprogramming that sustains increased cell proliferation (Ron-Harel et al., 2016) and that is reminiscent of the increased mitochondrial SGOCP activity seen in highly proliferative tumors (Ron-Harel et al., 2016). Mitochondrial biogenesis, and therefore the mitochondrial arm of the SGOCP, is severely impaired in tumor-infiltrating lymphocytes, which compromises their antitumor capacity (Scharping et al., 2016). Importantly, diet-derived serine reportedly impairs the pathogen-specific expansion of mouse T cells in vivo, indicating that serine-deprivation regimens, as a form of cancer therapy, could backfire by promoting immunosuppression (Ma et al., 2017). In the absence of serine, the proliferative capacity of T cells can be restored by the formate released in vivo by some tumor cells, although this is insufficient to maintain the tumor-infiltrating lymphocyte's tumoricidal function (Meiser et al., 2018). The innate immune system can be also controlled by the SGOCP activity. For example, serine catabolism from de novo–made serine potentiates IL-1β release by macrophages upon LPS stimulation in vivo and in an inflammasome-independent manner (Rodriguez et al., 2019).

Fibroblast activation, which involves major metabolic reprogramming, plays a crucial role in cancer progression (Gascard and Tlsty, 2016). p62, a multidomain signaling adaptor protein and autophagy chaperone, is a master regulator of the metabolic reprogramming that occurs in cancer-associated fibroblasts (CAFs; Linares et al., 2017; Reina-Campos et al., 2018; Valencia et al., 2014). Its loss decreases mTORC1 activity and impairs the metabolic detoxification potential of mouse fibroblasts, leading to increased IL-6 secretion and a protumorigenic inflammatory environment (Valencia et al., 2014). Thus, loss of p62 during fibroblast activation reduces the SGOCP activity, under nutrient-replete conditions, resulting in impaired serine and glycine synthesis through decreased mTORC1 activity that leads to lower amounts of reduced glutathione (Valencia et al., 2014). These observations have important consequences for tumor growth aided by CAFs under nutrient-rich conditions. However, tumor growth often occurs in nutrient-poor conditions in the TME (Finicle et al., 2018). In this context, p62-deficient fibroblasts are more resistant to glutamine deprivation because they up-regulate an ATF4-dependent signature that promotes cell survival through anaplerosis driven by the activation of pyruvate carboxylase (Linares et al., 2017). This enables p62-deficient fibroblasts to increase their survival potential and to support tumor cell proliferation by providing asparagine to the tumor cell. Asparagine is an alternative source of nitrogen in the absence of glutamine and is produced by an ATF4-dependent increase in asparagine synthase expression (Linares et al., 2017). These results are of relevance to human cancer because poorly fed tumors can metabolically hijack their surrounding stroma, which is then reprogrammed to provide nutrients and to sustain tumorigenesis. How tumors instruct CAFs to become nutrient suppliers remains unclear, but the process requires the down-regulation of p62 in CAFs, in response to as-yet-undefined signals emanating from the cancer epithelium.

An additional role for PHGDH in the TME involves endothelial cells of the tumor vasculature. PHGDH inhibition in these cells leads to lethal vascular defects due to increased oxidative stress and mitochondrial dysfunction caused by deficient nucleotide and heme synthesis (Vandekeere et al., 2018). Additionally, MTHFD2 in endothelial cells promotes the synthesis of purine-derived nucleotides in response to oxidized phospholipids and is required to synthesize glycine and to sustain angiogenesis (Hitzel et al., 2018). These SGOCP roles in endothelial cells support the rationale of targeting the pathway's enzymes to reduce angiogenesis and thus tumor growth.

Pending questions and potential new therapeutic opportunities

We now have a comprehensive view of the transcriptomic and metabolomic landscape of the SGOCP in human tumors, which allows us to identify specific and context-dependent relevant metabolic nodes for therapeutic intervention. In addition, several major supply routes that contribute to one-carbon units have been mapped, providing an unprecedented understanding of how this metabolic network is transcriptionally regulated and compartmentalized. However, none of these advances appear to have contributed to improve the antifolate chemotherapy pioneered by Sidney Farber and colleagues >70 yr ago. It is expected that new studies and more potent and specific chemical compounds could potentially offer much-needed breakthroughs in the targeting of this pathway in cancer.

An important line of research consists of finding new metabolic vulnerabilities that increase the specificity and sensitivity of established therapies. Examples include the inhibition of alternative sources of THF species, such as the catabolism of histidine or glycine by FTCD and GLDC, respectively, to increase the sensitivity of tumor cells to methotrexate (Kanarek et al., 2018; Newman et al., 1983; Zhang et al., 2012; Fig. 2). Conversely, methotrexate could be used to treat Wnt-driven malignancies by suppressing SAM levels and blocking arginine methylation (Albrecht et al., 2019). Similarly, inhibiting the synthesis or utilization of SAM could curtail acquired resistance by preventing transdifferentiation mechanisms and by effectively removing TICs that might cause cancer recurrence (Reina-Campos

et al., 2019; Visvader and Lindeman, 2008; Wang et al., 2019). The dependence of key enzymes on certain cofactors can also be exploited to support established therapies. For example, PHGDH's catalytic activity requires the cofactor NAD^+, which is partially supplied by the catabolism of tryptophan and kynurenine in specific cell types (Houtkooper et al., 2010), and by salvage routes from nicotinamide that are common to all tissues (Cantó et al., 2015). Inhibitors of the NAD^+ salvage pathway have been investigated as cancer therapeutics, but their efficacy has been limited (Burgos, 2011). However, breast cancer cells that overexpress PHGDH are vulnerable to NAD^+ salvage pathway inhibitors, such as those targeting nicotinamide phosphoribosyltransferase (Murphy et al., 2018).

It is now apparent that dietary interventions have profound effects on tumor metabolism and could be used to potentiate existing therapies (Maddocks et al., 2017). The cytoprotective actions of p53 in serine-depleted conditions indicate that p53-null tumors could be vulnerable to serine deprivation (Maddocks et al., 2013). However, high serine concentrations have been reported in the TME (Goveia et al., 2016), making it unclear whether serine deprivation could be exploited clinically as a vulnerability. Perhaps, serine deprivation could be achieved in combination with PHGDH inhibition together with the dietary restriction of serine. In fact, the dietary restriction of specific amino acids, such as serine and methionine, has been suggested as a potential coadjuvant therapy for cancer treatment. These diets impose a strain on metabolic networks, such as the SGOCP, which have to rewire their enzymatic routes to compensate for the deficient amino acid (Gao et al., 2019). This opens up vulnerabilities that could be exploited therapeutically, such as an impaired antioxidant response that induces DNA damage, in situations of dietary serine deprivation (Maddocks et al., 2017). However, the dietary restriction of SGOCP substrates, such as serine, choline, or methionine, needs to be approached with caution due to their possible toxic effects for normal organs. In fact, choline and methionine deprivation can induce fatty liver and HCC (Ghoshal et al., 1983). Also, the systemic deficiency of these amino acids could impact the SGOCP's activity in immune cells and impair key antitumor immune functions (Ron-Harel et al., 2016). Additionally, amino acid deprivation might engage compensatory mechanisms that sustain anabolism and proliferation, accelerating the emergence of therapy resistance. Thus, finding context-dependent metabolic dependencies could help to reveal those tumor types that might benefit from existing approved therapies and that could be more easily repurposed for new applications.

An additional line of research is focused on finding new actionable metabolic drivers of tumorigenesis. PHGDH is being actively pursued as a key metabolic vulnerability in tumors, as previously discussed (Mullarky et al., 2016; Pacold et al., 2016; Reina-Campos et al., 2019; Wang et al., 2017). For example, PHGDH inhibition is effective in the context of HIF2α-deficient clear cell renal cell carcinoma (Yoshino et al., 2017). However, the availability of serine in vivo, compensatory pathways, and other mechanisms of resistance are current barriers to the design of effective PHGDH inhibitors. Another key enzyme in the SGOCP, SHMT2, is a potential point of vulnerability and a driver of tumorigenesis, at least in diffuse large B cell lymphoma (Ducker et al., 2017; Zhang et al., 2012). This is because diffuse large B cell lymphoma is highly dependent on SHMT activity due to impaired glycine import (Ducker et al., 2017). MTHFD2 is another appealing target to prevent the acquisition of resistance (Gustafsson et al., 2017). As such, novel MTHFD2 inhibitors have been generated recently (Nishimura et al., 2019).

An alternative to inhibiting specific SGOCP enzymes is to target the pathway's upstream regulators, as a more potent mechanism to coordinately block the SGOCP. In this regard, ATF4 is a promising therapeutic target that could potentially inhibit important cell-autonomous and non–cell-autonomous mechanisms of survival (Linares et al., 2017). However, no small molecules have been reported that target ATF4 or, to our knowledge, RNA- or peptide-based inhibitors (Singleton and Harris, 2012). An even broader inhibition could be achieved by targeting the epigenetic program that maintains SGOCP gene activation via the H3K9 methyltransferase, G9A. Promising results have been generated recently using the G9A inhibitor BIX01294, which causes cell death through in vivo serine deprivation (Chen et al., 2017; Ding et al., 2013).

Inhibiting flux through the SGOCP in tumor cells is a daunting task, not least because the pathway is precisely designed to have built-in mechanisms to bypass roadblocks. However, the potent and irreversible inhibition of its key enzymes produces serious cytotoxic effects in many cancer cells. Priorities for future research to address this important question should therefore include finding the generation of more potent and selective chemical inhibitors and further transcriptomic and proteomic analysis of tumors to expose context-dependent vulnerabilities that can be effectively targeted. Such advances will be possible only through our increased understanding of this complex pathway.

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