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Cell-surface G-protein-coupled receptors for tumor-associated metabolites: A direct link to mitochondrial dysfunction in cancer

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Abstract

Mitochondria are the sites of pyruvate oxidation, citric acid cycle, oxidative phosphorylation, ketogenesis, and fatty acid oxidation. Attenuation of mitochondrial function is one of the most significant changes that occurs in tumor cells, directly linked to oncogenesis, angiogenesis, Warburg effect, and epigenetics. In particular, three mitochondrial enzymes are inactivated in cancer: pyruvate dehydrogenase (PDH), succinate dehydrogenase (SDH), and 3-hydroxy-3-methylglutaryl CoA synthase-2 (HMGCS2). These enzymes are subject to regulation via acetylation/deacetylation. SIRT3, the predominant mitochondrial deacetylase, directly targets these enzymes for deacetylation and maintains their optimal catalytic activity. SIRT3 is a tumor suppressor, and deacetylation of these enzymes contributes to its biological function. PDH catalyzes the oxidative decarboxylation of pyruvate into acetyl CoA, SDH oxidizes succinate into fumarate, and HMGCS2 controls the synthesis of the ketone body β -hydroxybutyrate. As the activities of these enzymes are decreased in cancer, tumor cells accumulate lactate and succinate but produce less amounts of β -hydroxybutyrate. Apart from their role in cellular energetics, these metabolites function as signaling molecules via specific cell-surface G-protein-coupled receptors. Lactate signals via GPR81, succinate via GPR91, and β -hydroxybutyrate via GPR109A. In addition, lactate activates hypoxia-inducible factor HIF1 α and succinate promotes DNA methylation. GPR81 and GPR91 are tumor promoters, and increased production of lactate and succinate as their agonists drives tumorigenesis by enhancing signaling via these two receptors. In contrast, GPR109A is a tumor suppressor, and decreased synthesis of β -hydroxybutyrate as its agonist suppresses signaling via this receptor, thus attenuating the tumor-suppressing function of GPR109A. In parallel with the opposing changes in lactate/succinate and β -hydroxybutyrate levels, tumor cells upregulate GPR81 and GPR91 but downregulate GPR109A. As such, these three metabolite receptors play a critical role in cancer and represent a new class of drug targets with selective antagonists of GPR81 and GPR91 for cancer treatment and agonists of GPR109A for cancer prevention.

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Keywords

Tumor-specific metabolism; Mitochondrial dysfunction; Warburg effect; Oncometabolites; Lactate and succinate; Epigenetics; Ketogenesis; Ketone bodies; Metabolite receptors; GPR81 and lactate; GPR91 and succinate; GPR109A and β -hydroxybutyrate; Butyrate and colonic bacteria

1. Introduction

In recent years there has been a renaissance in the field of cancer biology with the realization that tumor cells significantly modify their metabolism by reprogramming specific metabolic pathways. This is an interesting turn of events in the field because until recently the thrust in cancer research has been on oncogenes and tumor suppressors; but the possibility that the signaling pathways associated with these oncogenes and tumor suppressors impact oncogenesis, tumor growth, and metastasis at least partly by differentially regulating specific metabolic pathways did not receive much attention. Even the Warburg effect, which described almost nine decades ago an unusual enhancement of glycolysis in tumor cells even in the presence of adequate oxygen (aerobic glycolysis), was largely ignored by cancer biologists. Now all of a sudden there has been a burst of interest in tumor-specific metabolic pathways as potential drivers of tumor growth and metastasis and also as potential new drug targets for cancer therapy. The terms “glutaminolysis”, “glutamine addiction”, “reductive carboxylation” and “oncometabolites” have become very common in the vocabulary of cancer research.

Metabolic reprogramming in tumor cells optimizes utilization of various metabolic intermediates to drive cell proliferation and counteract cell death and apoptotic pathways. In the process, tumor cells generate specific metabolites at levels more than normal cells; many of these metabolites not only accumulate inside the tumor cells to unusually high levels but also get secreted, thus increasing their levels in tumor microenvironment. Understandably, as not all metabolic pathways are accelerated in tumor cells, some metabolites are generated at less than normal levels. The current review focuses on three specific metabolites, namely lactate, succinate, and β -hydroxybutyrate. Lactate and succinate are produced at higher than normal levels in tumor cells whereas β -hydroxybutyrate at lower than normal levels. Are the changes in the levels of these metabolites simply a bystander effect resulting from metabolic reprogramming in tumor cells? Or do these metabolites influence growth and proliferation of tumor cells? It would make sense if those metabolites that are elevated in tumor cells support and promote tumor growth whereas those metabolites that are reduced in tumor cells interfere with tumor growth. There is convincing evidence that this indeed is the case. Lactate and succinate promote tumor growth whereas β -hydroxybutyrate suppresses tumor growth. What is even more surprising is the discovery that these metabolites actually function as signaling molecules via specific cell-surface receptors and intracellular receptors to elicit their biological effects. The identification of cell-surface receptors for lactate, succinate, and β -hydroxybutyrate came from areas of research unrelated to cancer, but the currently increasing attention to these receptors in cancer biology is understandable based on the fact that the agonists for these receptors are tumor-specific metabolites. All three receptors, now recognized as metabolite receptors, are G-protein-coupled: GPR81 (also

known as Hydroxy Carboxylic Acid Receptor 1 or HCAR1) for lactate, GPR91 for succinate, and GPR109A (also known as Hydroxy Carboxylic Acid Receptor 2 or HCAR2; niacin receptor 1 or NIACR1). Of note are the findings that the expression of these receptors is altered in tumor cells in parallel with the changes in the levels of their agonists; GPR81 and GPR91 are upregulated and the levels of their respective agonists are increased in cancer. In contrast, GPR109A is downregulated and the levels of its agonist are decreased in cancer. The purpose of this review is to summarize the metabolic and regulatory pathways that lead to an increase in lactate and succinate production and a decrease in β -hydroxybutyrate production in tumor cells, and relate these changes to cellular effects of these three metabolites via their respective receptors.

2. Mitochondrial dysfunction and cancer

2. 1. Warburg effect

Mitochondria are the powerhouse in mammalian cells because this is where most of the metabolic energy is generated for use by the entire cell. It is also the place where many metabolic pathways occur, including citric acid cycle, oxidative phosphorylation, β -oxidation of fatty acids, ketogenesis, and some portions of the pathways related to urea cycle, heme synthesis and gluconeogenesis. The citric acid cycle and β -oxidation generate most of NADH and FADH₂ in the mitochondrial matrix, and oxidative phosphorylation that occurs in the innermitochondrial membrane couples oxidation of these reducing equivalents to reduction of molecular oxygen into water and converts the energy released in the reaction into ATP. The reduction of O₂ into H₂O is a four-step process in which four electrons are added in sequence with intermediate oxygen radicals such as superoxide (O₂•), peroxide (O₂²⁻), and oxygen radical (O•). Even though the four-step reduction produces the innocuous molecule H₂O when the reaction goes to completion, partially reduced intermediates of oxygen are generated to some extent in the electron transport chain, first in the form of superoxide, which then is subsequently converted into hydrogen peroxide (H₂O₂), and hydroxyl radical (OH•). The generation of superoxide occurs mostly at the level of coenzyme Q, involving complex I and complex III. These partially reduced intermediates of oxygen are highly reactive; they damage proteins, lipids, and DNA/RNA and compromise their biological functions. About 1–2% of oxygen consumed in the body goes through this pathway to generate reactive oxygen species (ROS); continuous exposure of the cells to ROS interferes with cellular functions and contributes to aging and death. If aging and consequent death are considered as diseases, then these can be called mitochondrial diseases because ROS generated in these organelles initiate the aging process. This highlights the paradox associated with mitochondrial function: it is necessary for cell survival as the site of production of the biological energy ATP that is obligatory for life but it is also the site of ROS production that ultimately causes cell death.

Given this paradox, tumor cells face a conundrum with regard to mitochondria. To support the high rate of proliferation, tumor cells need more ATP than normal cells, but reliance on mitochondria for this increased demand for energy also poses the risk of enhanced exposure to ROS. Tumor cells seem to circumvent this obstacle to their immortality by suppressing mitochondrial function and hence ROS production but generating ATP at increasing rates

from glycolysis in cytoplasm. As glycolysis does not use oxygen, it does not produce ROS. In normal cells, the rate of glycolysis is suppressed in the presence of oxygen because ATP generated by oxidative phosphorylation is a potent inhibitor of phosphofructokinase-1 (PFK1), the rate-limiting enzyme in glycolysis. But in tumor cells, glycolysis is accelerated even in the presence of oxygen, thus providing the basis for the term “aerobic glycolysis”, which is also referred to as the “Warburg effect” [1–5]. The key features of the Warburg effect in tumor cells include not only the accelerated glycolysis even under normoxic conditions but also the conversion of glucose into lactate, which occurs in normal cells only under hypoxic conditions. Owing to this mitochondrial dysfunction in tumor cells, the abilities of these cells to oxidize pyruvate, the end product of glycolysis, and NADH, the product of the reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase, are compromised. This poses a problem to tumor cells because if NADH is not oxidized to generate NAD^+ , the activity of glyceraldehyde-3-phosphate dehydrogenase will cease. This necessitates the conversion of pyruvate into lactate, catalyzed by lactate dehydrogenase, which oxidizes NADH into NAD^+ . Thus, lactate production as the end product of aerobic glycolysis in tumor cells is obligatorily coupled to Warburg effect. Lactate is not just a byproduct of dysregulated metabolism in tumor cells but actually functions as a signaling molecule to promote tumor growth as detailed later in this review. The generation of lactate in tumors is not only the result of the Warburg effect but could actually be the purpose of the Warburg effect to drive carcinogenesis and cancer growth [6].

2. 2. Reverse Warburg effect

Recent studies have shown that the Warburg effect (i.e., aerobic glycolysis and lactate production) may not be the characteristic of all tumor cells within a tumor [7–9]. This phenomenon might be present only in some tumor cells, primarily located at a far distance from blood vessels, and also in some stromal cells (e.g., cancer-associated fibroblasts). Other tumor cells, particularly those located in close proximity to blood vessels, might actually perform oxidative metabolism and utilize lactate, which is produced by their neighbors. As the production of lactate in some tumor cells and stromal cells is linked to Warburg effect, the utilization of lactate in other tumor cells capable of oxidative metabolism is referred to as the “Reverse Warburg effect.”

2. 3. Drivers of Warburg effect

What are the changes in tumor cells that fuel the Warburg effect? Why would tumor cells generate lactate even in the presence of oxygen? Warburg hypothesized that this phenomenon occurs due to mitochondrial malfunction [1]. Recent literature reviews provide experimental evidence that this hypothesis is indeed correct [10, 11]. Tumor cells exhibit alterations in mitochondrial biogenesis, mitophagy, and mitochondrial fusion and fission, all resulting in mitochondrial dysfunction. In addition, there are also genetic defects in mitochondrial DNA and nuclear DNA that compromise the functions of various proteins involved in oxidative phosphorylation [12–15]. However, the effective conversion of pyruvate into lactate resulting in the regeneration of NAD^+ does not explain entirely how glycolysis is accelerated despite the malfunction of mitochondria. There are several additional mechanisms that facilitate the Warburg effect in tumor cells. This includes enhanced glucose entry into cells, increased activities of glycolytic enzymes in the

cytoplasm, and metabolite changes in cells that relieve ATP-dependent inhibition of PFK1 [16, 17]. The entry of glucose into tumor cells is accelerated by upregulation of the facilitative glucose transporter GLUT1 (SLC2A1) as well as the active Na⁺-coupled glucose transporter SGLT1 (sodium/glucose co-transporter 1; SLC5A1) [2]. The oncogenes c-Myc and H-Ras along with hypoxia-inducible factor HIF1 α induce the expression of GLUT1 [18, 19] and growth factor signaling with downstream effectors Akt (a serine/threonine kinase) and PI3K (phosphoinositide-3-kinase) promote the translocation of preformed GLUT1 from intracellular vesicles to plasma membrane [20]. The upregulation of GLUT1 in tumor cells provides the basis for the use of non-metabolizable glucose analog ¹⁸F-2-deoxyglucose, which is a substrate for GLUT1, as a probe to detect tumors in vivo by positron emission tomography. This analog enters tumor cells at a much faster rate than in normal cells due to overexpression of GLUT1 in the former; it then gets phosphorylated by hexokinases and accumulates in tumor cells as the phosphorylated product cannot go further in glycolysis. Recent studies have shown that the Na⁺-coupled glucose transporter SGLT1 is also overexpressed in certain cancers [21, 22] and, in addition, is stabilized in tumor-cell plasma membrane by EGF receptor independent of its kinase activity [23]. In addition to enhanced glucose entry, glycolysis is further facilitated by increased activity of most of the glycolytic enzymes, either by induction or by post-translational modification [16, 17]. Two more changes in tumor cells fuel the Warburg effect, namely the differential isoform abundance of lactate dehydrogenase and cellular levels of fructose-2,6-bisphosphate. Tumor cells upregulate the expression of LDH-A but suppress LDH-B, a process that involves c-Myc and HIF1 α for LDH-A and promoter hypermethylation for LDH-B [24–27]. As LDH-A preferentially catalyzes the conversion of pyruvate into lactate whereas LDH-B the conversion of lactate into pyruvate, the increase in the LDH-A/LDH-B ratio in tumor cells promotes lactate production. Finally, the issue of PFK1 inhibition by ATP has to be addressed. Uninterrupted occurrence of aerobic glycolysis requires continued activity of PFK1 even in the presence of ATP. Tumor cells do make more ATP than normal cells, not by mitochondrial oxidative phosphorylation but by cytoplasmic glycolysis. Nonetheless, PFK1 is at risk for inhibition by ATP because the mechanism of ATP generation is irrelevant to this inhibition. Tumor cells resolve this obstacle by increasing the cellular levels of fructose-2,6-bisphosphate, a potent allosteric activator of PFK1 that prevents ATP-dependent inhibition. This is achieved by activation of phosphofructokinase-2 (PFK2) via AKT-mediated phosphorylation, which results in increased production of fructose-2,6-bisphosphate [28].

However, as indicated above while discussing “reverse Warburg effect”, the mitochondrial malfunction is not the characteristic of all cancer cells in a tumor tissue. The cancer cells that have an adequate supply of oxygen might not suffer from defective mitochondrial function and hence are likely to be capable of optimal oxidative metabolic pathways such as the citric acid cycle and glutaminolysis. This heterogeneity among the cancer cells in terms of oxygen supply and mitochondrial function is receiving increasing attention in recent years highlighting the notion that there is no one specific metabolic signature that describes all cancer cells.

3. Lactate and succinate as oncometabolites

3. 1. Regulatory mechanisms involved in increased lactate production in cancer cells: increased LDH-A/LDH-B ratio is only a part of the story

Pyruvate is the end product of glycolysis in cytoplasm, and as glycolysis is revved up in tumor cells, pyruvate production also goes up. There are four possible fates for pyruvate in mammalian cells: (i) it can be converted into lactate in cytoplasm via lactate dehydrogenase, (ii) it can be transferred into mitochondrial matrix for further metabolism, (iii) it can be transaminated into alanine in cytoplasm or in mitochondria, and (iv) it can be carboxylated into oxaloacetate in mitochondria. The conversion into lactate has received the most attention with regard to tumor cell metabolism because it is well recognized that cancer is associated with lactate production and it is mechanistically explainable by the increase in LDH-A/LDH-B ratio. Under non-equilibrium conditions as it exists in cells, LDH-A facilitates the conversion of pyruvate into lactate whereas the LDH-B facilitates the reverse reaction; this is made possible because of the differences between the two isoenzymes in specific biochemical features. LDH-A exhibits higher affinity for pyruvate than LDH-B; LDH-A is also less sensitive than LDH-B to substrate inhibition caused by pyruvate. But pyruvate has to be made available for the lactate dehydrogenase to produce lactate; for this, the metabolism of pyruvate within the mitochondria has to be suppressed. The entry of pyruvate across the innermitochondrial membrane serves as the gatekeeper for its metabolism within the mitochondria. Pyruvate transfer into mitochondria occurs via a H^+ -coupled process mediated by a heterodimeric transporter complex consisting of MPC1 and MPC2 (mitochondrial pyruvate carrier 1 and 2) present in innermitochondrial membrane [29]. This transporter plays an important role in metabolic reprogramming in cancer cells; loss of its transport function promotes Warburg's metabolic phenotype and cancer growth [30–31]. Recent studies have highlighted the potential of this transporter as a drug target for cancer therapy [32, 33]. Once inside mitochondria, pyruvate is metabolized into acetyl CoA via pyruvate dehydrogenase complex (PDC) as the principal pathway (Fig. 1). PDC is almost an irreversible enzyme because of the high negative ΔG for the forward reaction towards acetyl CoA production. PDC is subject to regulation by different mechanisms. First, it is regulated by phosphorylation/dephosphorylation. PDC consists of three subunits: E1 α (pyruvate decarboxylase)-E1 β (a modulator of E1 α activity), E2 (dihydrolipoyl transacetylase), and E3 (dihydrolipoyl dehydrogenase); among these, E1 α is subject to regulation by post-translational modification involving phosphorylation. The unphosphorylated form is more active than the phosphorylated form. Phosphorylation is mediated by PDK1 (pyruvate dehydrogenase kinase 1), a cAMP-independent protein kinase, and the reverse reaction for dephosphorylation is mediated by PDP1 (pyruvate dehydrogenase phosphatase 1) [34, 35]. E1 α is also regulated by acetylation/deacetylation; the acetylation is mediated by ACAT1 (acetyl CoA acetyl transferase 1) and the deacetylation is mediated by SIRT3, a mitochondrial deacetylase that is dependent on NAD^+ [36]. This acetylation/deacetylation pathway co-operates with the phosphorylation/dephosphorylation pathway [37]. There could also be some role for protein succinylation in the regulation of PDC; but interestingly the activity of PDC is increased in the succinylated state, which is the opposite of what happens to its activity upon acetylation, and the desuccinylation is mediated by SIRT5, not by SIRT3 [38]. In tumor cells, the levels of the

phosphorylated form and the acetylated form of E1 α increase due to tumor-associated upregulation of PDK1 and down-regulation of SIRT3 (Fig. 1). The end result is a decrease in PDC activity, which consequently suppresses the conversion of pyruvate into acetyl CoA, thus channeling pyruvate into lactate dehydrogenase reaction to produce lactate.

3. 2. Release of lactic acid from tumor cells

While it is easily understandable that tumor cells generate lactic acid by accelerating aerobic glycolysis to prevent mitochondrial production of ROS, this metabolic switch poses a new problem: cellular acidification. Tumor cells have to protect themselves from lactic acid-induced acidification; they accomplish this goal by releasing lactate and H⁺ into extracellular medium using a transporter in the plasma membrane (Fig. 1). Among the several H⁺-monocarboxylate transporters belonging to the SLC16 family, MCT4 (monocarboxylate transporter 4; SLC16A3) appears to be primarily responsible for this process [39, 40]. MCT4 is a low-affinity transporter with a Michaelis constant for lactate in the range of 30–40 mM, a kinetic feature perfectly suitable for the release of lactate from tumor cells that generate massive amounts of this metabolite. HIF1 α , which plays a critical role in the maintenance of the Warburg metabolic phenotype in tumor cells resulting in lactic acid production, also is responsible for the induction of MCT4 in tumor cells to mediate the lactic acid efflux [41]. This is however not the sole mechanism utilized by tumor cells to maintain intracellular pH in the face of increased lactic acid production; the expression of several other transporters and enzymes that are capable of pH regulation is altered in tumor cells [42, 43]. The release of lactic acid from tumor cells creates an acidic microenvironment, which in itself might have important biological functions related to tumor invasion, angiogenesis, and metastasis. MCT1 is also induced in some tumor cells; this is a high-affinity transporter with a Michaelis constant for lactate in the range of 2–5 mM [39, 40]. It is suitable to mediate cellular uptake of lactic acid; accordingly, this transporter might be expressed selectively in tumor cells capable of oxidative metabolism so that lactic acid released from hypoxic tumor cells can be used by oxygen-replete tumor cells for cellular energy production.

3. 3. Changes in succinate metabolism in tumor cells

Succinate is an intermediate in citric acid cycle that occurs within the mitochondrial matrix. This metabolite hardly received any attention in the field of cancer biology until it was discovered that cellular levels of succinate go up in certain cancers and that it is an effective modifier of epigenetics relevant to carcinogenesis [44–46]. Succinate dehydrogenase (SDH) oxidizes succinate into fumarate in citric acid cycle. This enzyme is unique in many ways; it is the only enzyme that generates FADH₂ in the cycle, it is also a component of the electron transport chain where it is called “Complex II”, and among the five complexes in the electron transport chain and oxidative phosphorylation pathway, only Complex II has no component that is encoded by mitochondrial DNA. SDH consists of four subunits: SDH-A, SDH-B, SDH-C, and SDH-D. The activity of SDH is controlled by acetylation/deacetylation involving SIRT3 [47, 48] and also by iron-dependent regulation [49, 50] (Fig. 2). SDH-A is the target for acetylation by ACAT1 and deacetylation by SIRT3. The enzyme is more active in non-acetylated form than in acetylated form. As such, the mitochondrial deacetylase SIRT3 is obligatory for optimal activity of SDH. SIRT3 is also an iron-regulatory protein via

its ability to modulate ROS levels [50]. The electron transport chain is an important site of superoxide production; this ROS is detoxified by mitochondrial superoxide dismutase, an enzyme regulated by acetylation/deacetylation and whose catalytic activity is maintained at optimal levels by SIRT3-mediated deacetylation. Thus, SIRT3 plays a role in keeping ROS at low levels in cells. When cellular levels of ROS are reduced, the ability of the iron-regulatory protein 1 (IRP1) to interact with iron-responsive elements (IRE) in mRNA is compromised. SDH-B mRNA contains an IRE in the 5'-end, and binding of IRP1 to this site suppresses mRNA translation (Fig. 2). SIRT3 interferes with the interaction of IRP1 with IRE in SDH-B mRNA by reducing ROS levels and consequently promotes SDH-B protein translation. As such, the mitochondrial deacetylase SIRT3 has a dual role in keeping SDH in active state.

SIRT3 is downregulated in certain cancers as it functions as a tumor suppressor, though in some other cancers the opposite might be true [51, 52]. The tumor-suppressive function of SIRT3 is at least partly due to its ability to keep SDH in active state, thus reducing the cellular levels of succinate. When SIRT3 expression goes down as it happens in some cancers, the activity of SDH-A is reduced and the protein levels for SDH-B are decreased, consequently compromising the conversion of succinate into fumarate and increasing the cellular levels of succinate. Succinate is a divalent anion; as such it cannot leave the cell without involvement of specific transport mechanisms. There is little information available on the transporters responsible for the efflux of succinate in mammalian cells, including tumor cells. However, several transporters that are selective for organic anions do participate in the efflux of anionic metabolites by functioning as obligatory exchangers. This includes the organic anion transporters belonging to the *SLC22* gene family and the organic anion transporting polypeptides belonging to the *SLCO* (previously *SLC21*) gene family [53, 54]. It is highly possible that one or more of the transporters in these two gene families mediate the release of succinate from tumor cells into the extracellular medium (Fig. 2).

4. Intracellular actions of lactate and succinate as tumor promoters

4. 1. Lactate as a regulator of HIF1 α levels in cells

In normal cells (with the exception of erythrocytes), lactate production is a biomarker for hypoxia. Therefore, it would make sense if lactate functions as a signaling molecule to enhance angiogenesis and blood supply to the tissues that generate this metabolite. Two signaling pathways have been identified by which lactate promotes angiogenesis. First, lactate is an inhibitor of prolylhydroxylase 2 (PHD2), an enzyme that controls the turnover of HIF1 α [55]. PHD2 catalyzes the hydroxylation of proline residues in HIF1 α in an oxygen- and α -ketoglutarate-dependent manner. As the activity of PHD2 relies on the availability of O₂, this enzyme functions as an oxygen sensor. Under normoxic conditions, PHD2 uses O₂ to hydroxylate HIF1 α for subsequent ubiquitination by Von Hippel-Lindau protein complex and proteasomal degradation. Under hypoxic conditions, PHD2 is less active, thus preventing HIF1 α degradation. As lactate inhibits PHD2, HIF1 α levels increase in lactate-generating cells irrespective of whether these cells are under normoxic or hypoxic conditions. This is what happens in tumor cells. Increased production of lactate enhances the biological activities of HIF1 α , thus promoting angiogenesis among other multitude of

HIF1 α effects. Interestingly, these effects of lactate do not occur in tumor cells with the Warburg metabolic phenotype but in tumor cells with oxidative metabolism [55]. One possible explanation for this seemingly contradictory phenomenon is that even though tumor cells with the Warburg phenotype generate lactate, they effectively remove this metabolite via MCT4-mediated efflux. In contrast, tumor cells that exhibit oxidative metabolism take up lactate via MCT1, thus accumulating this metabolite inside the cells for energy production. There could be other additional, still reasonable and rational, explanations for this phenomenon.

4. 2. Lactate as a signaling molecule via an intracellular receptor

In addition to HIF1 α , there are other protein targets of PHD2. One of them is NDRG3 (N-Myc Downstream Regulated Gene 3). NDRG3 is also hydroxylated by PHD2 for subsequent ubiquitination and proteasomal degradation [56]. NDRG3 enhances angiogenesis and cell proliferation via its ability to bind c-Raf and activate Raf-ERK pathway. Lactate as an inhibitor of PHD2 protects this tumor promoter from proteasomal degradation. Interestingly, NDRG3 also functions as a receptor for lactate; its binding to lactate protects it from ubiquitination and subsequent degradation. As such, lactate increases the cellular levels of the tumor promoter NDRG3 by two independent mechanisms. NDRG3 functions as a tumor promoter and metastasis activator at least in some cancers [57, 58], and lactate as an enhancer of NDRG3 activity provides a mechanistic link between this tumor-associated metabolite and tumor growth and metastasis.

4. 3. Succinate as a regulator of HIF1 α and also a modifier of epigenetics

The relevance of succinate to carcinogenesis and tumor growth came to the forefront in the field of cancer biology with the observations that loss-of-function mutations in succinate dehydrogenase (SDH) are sufficient to drive certain cancers such as paraganglioma, renal cell carcinoma, and gastrointestinal stromal tumors [45, 59, 60]. A number of Fe²⁺ - and α -ketoglutarate-dependent dioxygenases play a critical role in HIF1 α turnover and also in methylation of DNA and histones. Prolylhydroxylases such as PHD2, which hydroxylates specific proline residues in HIF1 α to facilitate subsequent ubiquitination and proteasomal degradation, ten-eleven translocation (TET) enzymes, which hydroxylate 5-methylcytosine in DNA for subsequent removal of the methyl group, and Jumonji C domain-containing histone demethylases, also called lysine demethylases (KDMs), belong to this dioxygenase family. As α -ketoglutarate is structurally similar to succinate and also as the reactions catalyzed by these enzymes produce succinate as a product, succinate inhibits these enzymes by competing with α -ketoglutarate in catalysis and also by product inhibition [61]. Inhibition of PHDs suppresses proteasomal degradation of HIF1 α [62, 63], inhibition of TETs enhances promoter methylation in specific genes [64, 65], and inhibition of KDMs promotes histone lysine methylation [65]. As with lactate, accumulation of succinate in cells is also a biomarker of hypoxia and mitochondrial dysfunction. As such, the cells that are exposed to excess succinate, irrespective of the molecular process that leads to succinate accumulation, might perceive themselves to be in hypoxia; hence it makes sense that succinate serves as a signaling molecule to potentiate the function of HIF1 α .

5. Cell-surface receptors for lactate and succinate and their role in cancer

5. 1. Identity of GPR81 as the lactate receptor and its function as a tumor promoter

Recent studies have identified the cell-surface G-protein-coupled receptor GPR81 as the lactate receptor [66, 67]. According to the nomenclature recommended by the International Union of Basic and Clinical Pharmacology, GPR81 is called HCAR1 (Hydroxy Carboxylic Acid Receptor 1) [68]. This HCAR family consists of three members, and the physiological agonists for all of them are specific hydroxy carboxylic acids. The physiological agonist for GPR81 is L-lactate (L- β -hydroxy propionate). This receptor is coupled to $G_{i/q}$, and activation of the receptor results in decreased cellular levels of cAMP and increased cellular levels of Ca^{2+} . This receptor was first discovered in adipocytes and therefore the focus on this receptor has been mostly on its role as an inhibitor of lipolysis [69]. But the relevance of this receptor to cancer is receiving increasing attention in recent years. This is easily understandable because of the high levels of lactate in tumor microenvironment and the growing evidence that lactate elicits biological effects as a signaling molecule.

GPR81 is upregulated in most cancers (pancreas, colon, liver, breast, lung, cervix) [70–73], and in many cases the expression levels correlate with tumor growth and metastasis. Both in vitro and in vivo, activation of GPR81 with lactate promotes tumor growth. Silencing of the receptor leads to rapid death in cancer cells under culture conditions where the cells rely on lactate instead of glucose as the source of metabolic energy [70]. In cervical cancer cells, activation of the receptor with lactate promotes DNA repair and mediates chemoresistance [71]. Interestingly, the expression of the lactate transporters MCT1 and MCT4 is modulated by GPR81; exposure of pancreatic cancer cells to lactate in extracellular medium increases the expression of these two transporters, thus facilitating release of lactate from cancer cells [70]. GPR81 activation also promotes a malignant phenotype in cancer cells by enhancing angiogenesis via increased secretion of the pro-angiogenic factor amphiregulin [72]. This process involves the PI3K/AKT pathway coupled to decreased cellular levels of cAMP. These diverse biological effects of lactate mediated through GPR81 strongly indicate that the receptor is crucial for cancer cell survival, tumor growth, metastasis, and chemoresistance (Fig. 3).

5. 2. Pharmacological agonists for GPR81

Lactate is the only known endogenous agonist for GPR81; its affinity is in millimolar range (1–5 mM) [66, 67], but these are achievable concentrations under normal physiological conditions. Lactate levels in circulation are normally in the range of 1–2 mM, but can reach as high as 20 mM under conditions such as strenuous exercise. Pharmacological agonists have been identified that activate GPR81 at micromolar and nanomolar concentrations (Table 1) [74–76]. 3,5-Dihydroxybenzoic acid activates GPR81 with an EC_{50} value of ~150 μ M [74]. Replacement of the hydroxyl group at the third position with certain functional groups increases the potency markedly; 3-chloro-5-hydroxybenzoic acid is almost 10-fold more potent, with an EC_{50} value of ~15 μ M [75]. The most potent agonist of GPR81 to date is 4-methyl-N-(5-(2-(4-methylpiperazin-1-yl)-2-oxoethyl)-4-(2-thienyl)-1,3-thiazol-2-yl)cyclohexanecarboxamide with an EC_{50} value of ~60 nM [76]. A recent study has reported that the ketone body β -hydroxybutyrate is an antagonist of GPR81 [77]. As GPR81

signaling promotes tumor progression and metastasis, highly selective antagonists of this receptor might have therapeutic potential as anticancer agents.

5. 3. GPR81 and inflammation

There is a strong connection between inflammation and carcinogenesis; pro-inflammatory conditions can either promote or suppress cancer growth in a context-specific manner. Convincing evidence indicates that activation of GPR81 initiates anti-inflammatory signaling in pancreas, liver and uterus [78, 79]. In pancreas and liver, lactate abrogates tissue injury caused by Toll-like receptor and inflammasome-mediated inflammation both in vitro and in vivo via GPR81 expressed in macrophages [78]. The GPR81-dependent suppression of inflammation in the uterine tissue is related to parturition [79]. The onset of labor is initiated by inflammatory processes in uterus, and during labor uterine contraction generates high levels of lactate via anaerobic glycolysis. Subsequent activation of GPR81 by lactate then elicits anti-inflammatory effects on uterine tissue in a negative feedback manner to resolve inflammation. How the anti-inflammatory actions of GPR81/lactate signaling relate to cancer remains to be explored.

5. 4. Identity of GPR91 as the succinate receptor and its potential role in cancer

GPR91 was identified as the receptor for succinate in the kidney, principally expressed in macula densa cells [80]. Subsequent studies have uncovered a critical role for this receptor in blood pressure regulation via modulation of the renin-angiotensin system [81]. GPR91 activation elicits additional biological effects that could have relevance to cancer. Succinate is an important regulator of angiogenesis in the retina [82]. Though succinate stabilizes HIF1 α , which could increase angiogenesis, the activation of GPR91 by succinate promotes angiogenesis in a HIF1 α -independent manner [82]. The ERK1/2-COX-2/PGE2 signaling pathway seems to underlie the GPR91-induced release of vascular endothelial growth factor [83]. The succinate/GPR91 signaling is also a promoter of hematopoiesis in bone marrow [84]. As mentioned earlier, accumulation of succinate is a biomarker for hypoxia and mitochondrial functional deficiency; hence it seems logical that this metabolite has a positive effect on angiogenesis and hematopoiesis. Recent studies have shown that succinate plays a role liver fibrosis by activating stellate cells [85, 86]. With regard to inflammation, the succinate/GPR91 signaling pathway produces pro-inflammatory effects in contrast to the lactate/GPR81 signaling pathway [87, 88]. Though there are no reports in the literature to date on any direct link between GPR91 and cancer, the ability of this receptor to activate hematopoiesis, angiogenesis, inflammation, and tissue fibrosis strongly suggests that succinate is likely to function as a tumor promoter via GPR91. Of relevance to this issue are the observations that the expression of GPR91 is upregulated in response to iron overload as occurs in genetic diseases such as hemochromatosis [89, 90]. There is unequivocal evidence for chronic exposure to excess iron as a risk factor for cancer [91–93]. The increased expression of the succinate receptor in response to excessive iron could be one of the factors underlying the connection between iron overload and cancer. Recently pharmacological agonists have been identified (e.g., cis-epoxysuccinate) that activate the succinate receptor with potencies greater than that of succinate [94]. These new agonists represent useful experimental tools to investigate the function of the receptor and its relevance to cancer.

6. GPR109A as the receptor for butyrate and β -hydroxybutyrate and its connection to cancer

6.1. Identification of GPR109A as the receptor for butyrate in colon

GPR109A was first identified as the receptor for the B-complex vitamin niacin [94, 95]. Niacin has been in use for several decades as a lipid-lowering drug, reducing circulating levels of cholesterol, decreasing LDL and increasing HDL, but the molecular mechanism responsible for these pharmacological actions of niacin remained unknown. The discovery of GPR109A, a G-protein-coupled receptor associated with G_i , as the receptor for niacin explained how niacin worked. The receptor is expressed robustly in adipocytes, and activation of the receptor with niacin decreases cellular levels of cAMP, which inactivates the lipolytic enzyme hormone-sensitive lipase that is activatable by cAMP-dependent protein kinase [95, 96]. But niacin is only a pharmacological agonist for the receptor; circulating levels of niacin are never in the range sufficient to activate the receptor unless niacin is taken at pharmacological doses (normal plasma concentration, $< 0.1 \mu\text{M}$; EC_{50} value for activation of the receptor, $\sim 1 \mu\text{M}$). This raised the question as to the identity of physiological agonists for the receptor. Subsequent research led to the identification of β -hydroxybutyrate, the principal ketone body in circulation, as the endogenous agonist for the receptor [97]. The EC_{50} value for this endogenous ligand to activate the receptor is $\sim 700 \mu\text{M}$, almost three orders of magnitude less potent than niacin, but the circulating levels of this compound can reach as high as 2 mM under physiological conditions and as high as 20 mM under certain pathological conditions such as prolonged starvation or uncontrolled diabetes. In the same study which discovered β -hydroxybutyrate as the agonist, butyrate was also found to activate the receptor but at much higher concentrations (EC_{50} , $\sim 1.5 \text{ mM}$) [97]. Plasma levels of butyrate never go beyond micromolar concentrations; however, colonic lumen contains millimolar concentrations of this fatty acid, resulting from bacterial fermentation of dietary fiber, suggesting that butyrate could function as the endogenous agonist for the receptor if the lumen-facing apical membrane of colonic epithelial cells expresses the receptor. Subsequent studies have shown that GPR109A is indeed expressed in this membrane and that butyrate does activate the receptor at concentrations normally found in the colonic lumen [98, 99]. As the receptor is found on the lumen-facing membrane in colon cells, β -hydroxybutyrate present in the circulation has no relevance to the receptor in this tissue. These findings demonstrate that while the ketone body β -hydroxybutyrate is the endogenous agonist for GPR109A in non-colonic tissues, the bacterial metabolite butyrate is the endogenous agonist for the receptor in colon.

6. 2. GPR109A as a tumor suppressor in colon and its relevance to dietary fiber and colonic bacteria

Dietary fiber and colonic bacteria play an important role in protecting colon from inflammation and carcinogenesis, and short-chain fatty acids, which are the products of bacterial fermentation of dietary fiber in colonic lumen, are thought to be the principal mediators of this protection [100]. The short-chain fatty acids in colonic lumen primarily consist of acetate, propionate, and butyrate in a ratio of 6:3:1 with the total concentration of 50–100 mM. As butyrate is an inhibitor of histone deacetylases, the major focus on the

protective effects of dietary fiber against colitis and colon cancer has been on butyrate-induced epigenetic changes in colonic epithelium as the driver of this phenomenon [2, 43, 101, 102]. The discovery of GPR109A as the butyrate receptor in colonic epithelial cells shifted the focus significantly to butyrate as a signaling molecule. Several studies have now demonstrated that activation of GPR109A by butyrate suppresses inflammation and protects against carcinogenesis in colon [98, 103, 104], and the findings of these studies have been reviewed in recent years [105–107].

The expression of GPR109A is downregulated in human colon cancer and in mouse models of colon cancer [98]. Most colon cancer cells express low levels of the receptor, and the molecular mechanism for the low expression in cancer cells involves promoter hypermethylation. Ectopic expression of GPR109A in colon cancer cells followed by exposure to GPR109A agonists such as niacin and butyrate causes apoptosis. These studies strongly indicate that GPR109A functions as a tumor suppressor in colon. These in vitro findings are corroborated by in vivo data. *Gpr109a*-null mice show increased susceptibility to experimental colitis and colon cancer [103, 104]. As the involvement of the receptor in immune function is very robust, detailed studies have been done on how GPR109A signaling impact mucosal immune system. The receptor is expressed not only on colonic epithelial cells where it is located on the lumen-facing apical membrane thus having access to luminal butyrate as its agonist but also in antigen-presenting immune cells such as macrophages and dendritic cells [103]. Both non-hematopoietic and hematopoietic components contribute to the protection against inflammation and carcinogenesis. When exposed to GPR109A agonists, the biology of dendritic cells and macrophages is significantly altered with increased ability to convert naïve T cells into IL-10-producing T cells and immunosuppressive Tregs [103]. Moreover, GPR109A signaling is essential for butyrate-mediated induction of IL-18 in colonic epithelial cells. Pharmacological doses of niacin in drinking water potently suppress the disease progression in experimental models of colitis and colon carcinogenesis [103]. These studies show that GPR109A expressed in the colonic epithelium provides a direct link between colonic bacteria and the host; the bacterial fermentation product butyrate serves as the messenger in this bacteria-host communication. Of relevance to this are the findings that colonic bacteria control the expression of the receptor in colon cells [99]. Germ-free mice show markedly reduced expression of the receptor in colonic epithelial cells, but when germ-free mouse colon is re-colonized, the expression levels come back to normal. This shows an active role for colonic bacteria in controlling gene expression in the colon.

6. 3. β -Hydroxybutyrate as the potential agonist for GPR109A in mucosal immune cells

While it is obvious that the bacteria-derived butyrate is the physiological agonist for GPR109A expressed on the luminal membrane of colonic epithelial cells, this bacterial metabolite is not likely to be the agonist for the receptor expressed in mucosal immune cells present in colonic lamina propria. These immune cells do respond in vitro to exposure to butyrate or niacin, but this merely indicates the involvement of GPR109A. Even though colonic epithelial cells are exposed to millimolar concentrations of butyrate in the lumen and avidly take up this bacterial metabolite, most of it gets metabolized within the cells. Only a small fraction of butyrate leaves the colonic epithelial cells as evident from the low levels of

this metabolite in portal blood (~50 μM). These concentrations are not sufficient to activate GPR109A. We speculate that β -hydroxybutyrate is the physiological agonist for the receptor in mucosal immune cells. In a fed state, the circulating levels of β -hydroxybutyrate are approximately 50–100 μM , but these levels increase considerably in between meals. Furthermore, colonic epithelial cells are capable of ketogenesis, having all the enzymatic machinery to synthesize β -hydroxybutyrate [108, 109]. The role of liver as a ketogenic organ is well known, but the fact that colon is also a ketogenic organ is not widely recognized. We speculate that colonic epithelial cells synthesize β -hydroxybutyrate, presumably using the bacterial metabolite butyrate as the starting material, and release the ketone body into portal circulation. The monocarboxylate transporter MCT1 (SLC16A1) expressed in the basolateral membrane of the epithelial cells is likely to be responsible for the export of β -hydroxybutyrate. Consequently, the antigen-presenting macrophages and dendritic cells present in colonic lamina propria would be exposed to relatively higher concentrations of this agonist than present in systemic circulation. Interestingly, the expression of the rate-limiting enzyme in the synthesis of β -hydroxybutyrate, 3-hydroxy-3-methyl glutaryl CoA synthase 2 (HMGCS2), is repressed by the oncogene c-Myc [109]. HMGCS1 is a cytosolic enzyme involved in cholesterol biosynthesis whereas HMGCS2 is a mitochondrial enzyme involved in ketogenesis. The promoter region of the gene coding for HMGCS2 contains a Sp1-binding site that binds the transcription factor Miz-1 and transactivates the gene, but c-Myc blocks the transactivating function of Miz-1 (Fig. 4). The expression of HMGCS2 is suppressed in c-Myc-dependent colon and rectal cancers [109], suggesting that the capacity of colonic epithelial cells to generate this GPR109A agonist is compromised in colon cancer. This would interfere with the ability of the antigen-presenting cells in the lamina propria to induce Tregs and IL-10-producing T cells and create a pro-inflammatory environment that would be conducive for carcinogenesis. HMGCS2 is also subject to regulation by post-translational modification involving acetylation/deacetylation (Fig. 4). SIRT3, the mitochondrial NAD^+ -dependent deacetylase, is responsible for the maintenance of HMGCS2 in the deacetylated state, thus keeping the enzyme in optimally active form [110]. *Sirt3*-null mice have markedly reduced capacity for ketogenesis. All these findings point to a tumor-suppressive role for β -hydroxybutyrate in colon.

6. 4. GPR109A as a tumor suppressor in non-colonic tissues

The findings that β -hydroxybutyrate is an agonist for GPR109A suggest that the tumor-suppressive function of the receptor might not be restricted to colon because of the presence of this agonist in systemic circulation and also because of the expression of the receptor in a number of non-colonic cell types. Recent studies have extended the tumor-suppressive function of the receptor beyond colon; GPR109A suppresses mammary tumorigenesis in a mouse model of breast cancer [111]. The receptor is expressed in normal mammary tissue, and irrespective of the estrogen receptor status, the receptor is silenced in breast cancer. Most of the breast cancer cell lines, either positive or negative for estrogen receptor, do not express GPR109A. As seen with colon cancer cell lines, ectopic expression of the receptor in breast cancer cell lines leads to apoptosis upon exposure to receptor agonists. The tumor-suppressive function of the receptor is also demonstrable in vivo using MMTV-Neu mouse model of breast cancer; deletion of *Gpr109a* in MMTV-Neu mouse promotes tumor incidence and tumor growth, triggers early onset of tumorigenesis, and potentiates lung

metastasis. Transcriptome analysis has shown that activation of GPR109A signaling in breast cancer cells leads to suppression of the genes involved in cell survival.

The strong evidence for the tumor-suppressive function of GPR109A in a non-colonic tissue is important as it highlights the potential relevance of circulating levels of β -hydroxybutyrate to cancer prevention. There are a number of physiological and dietary conditions that can elevate the levels of β -hydroxybutyrate in circulation, examples of which include caloric restriction and ketogenic diets. As the expression of GPR109A is silenced in cancer, activation of the receptor with β -hydroxybutyrate can only be used as a cancer prevention strategy. Based on the data available from the involvement of the receptor expression and signaling in colon cancer and breast cancer, we conclude that both immune and non-immune mechanisms contribute to cancer prevention mediated by GPR109A signaling (Fig. 5). This also opens up new avenues for chemoprevention as a number of high-affinity pharmacological agonists have been identified for GPR109A (Table 1). Niacin is a B-complex vitamin that can be taken at pharmacological doses to increase the circulating levels sufficient to activate the receptor in immune cells and in systemic organs. This vitamin has been in wide use as a lipid-lowering agent, but in the light of the recent findings with GPR109A as a tumor suppressor, niacin might have potential as a cancer prevention drug. Some studies have however shown that chronic use of niacin at pharmacological doses increases the risk of diabetes via GPR109A-dependent suppression of insulin secretion [112, 113]. It has to be noted however that niacin influences a broad spectrum of biological processes in mammalian cells in addition to its role as an agonist for GPR109A. Important among them is the role of niacin as a precursor for NAD^+ and NADP^+ . These coenzymes are critical for several metabolic pathways such as glycolysis, gluconeogenesis, fatty acid synthesis, β -oxidation, cholesterol synthesis, and hexose monophosphate shunt. Therefore, it is difficult to determine the exact mechanism by which chronic use of niacin leads to dysregulation of glucose control. Other pharmacological agonists of GPR109A might not have this undesirable side effect. Nonetheless, any potential use of GPR109A agonists for cancer prevention needs caution. The same is true with caloric restriction and ketogenic diets that increase the circulating levels of the endogenous agonist β -hydroxybutyrate for GPR109A. There have been some reports that ketogenic diets might actually promote cancer by providing β -hydroxybutyrate as a metabolic fuel for cancer cells [114]. Obviously additional studies are needed to understand the differential contribution of this ketone body as a GPR109A agonist (tumor suppressor) and as a metabolic fuel (tumor promoter).

6. 5. Pharmacological agonists for GPR109A

The recognition of GPR109A as a tumor suppressor is fairly recent, but the role of this receptor in inhibiting lipolysis in adipocytes and hence in decreasing circulating levels of free fatty acids and LDL has been known since the discovery of the receptor. As such, there is considerable interest in this receptor as a drug target for dyslipidemia. This has resulted in focused efforts in identifying selective and high affinity agonists for this receptor, both in academia and in pharmaceutical industry. The compounds that have been identified thus far as the activators of GPR109A and their EC_{50} values are listed in Table 1. Recent reviews have summarized the various features of these agonists [69, 115].

7. SIRT3 and its link to the metabolite receptors and cancer

Mitochondrial function is fundamentally linked to lactate, succinate, and β -hydroxybutyrate. Deficiency of mitochondrial function impacts circulating levels of all three metabolites. SIRT3 is the principal NAD^+ -dependent deacetylase critical for optimal mitochondrial function. The enzymatic activity of SIRT3 is responsible for the maintenance of optimal activities of pyruvate dehydrogenase, succinate dehydrogenase, and HMGCS2, which is essential for normal oxidation of lactate and succinate and for normal production of β -hydroxybutyrate. As lactate and succinate promote cancer via their respective receptors GPR81 and GPR91 whereas β -hydroxybutyrate protects against cancer via its receptor GPR109A, SIRT3 functions are associated with tumor suppression. SIRT3 deficiency elicits the opposite effects by increasing cellular and circulating levels of the tumor promoters lactate and succinate and by compromising the generation of the tumor suppressor β -hydroxybutyrate. The functional link between SIRT3 and the metabolite receptors GPR81, GPR91, and GPR109A highlights the potential of SIRT3 as a drug target for cancer prevention and cancer treatment.

8. Conclusions

The functional relevance of the tumor-associated metabolites to cancer progression is just beginning to be understood. The recognition of lactate and succinate as oncometabolites reveals only a part of the story linking these metabolites to cancer growth. There is increasing evidence in recent years that these two metabolites also function as tumor-promoting signaling molecules via specific cell-surface G-protein-coupled receptors. The circulating levels of lactate and succinate and the expression of their respective receptors are coordinately increased in tumors to facilitate their role as tumor promoters. As such, selective high-affinity antagonists for these receptors have potential as novel drugs for cancer treatment. The ketone body β -hydroxybutyrate seems to have complex relationship to cancer. Activation of GPR109A by this metabolite suppresses tumor growth, and accordingly the capacity of tumor cells to generate β -hydroxybutyrate is decreased, at least in c-Myc-driven cancers. Congruent with the findings that GPR109A signaling leads to tumor suppression, the expression of the receptor is silenced in cancer. As such, selective high-affinity agonists of this receptor have potential for cancer prevention.

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Pyruvate Dehydrogenase Complex (PDC)

Fig. 1. Regulation of pyruvate dehydrogenase complex in mitochondria by post-translational modification and its relevance to cancer

(A) Reaction mediated by pyruvate dehydrogenase complex (PDC). (B) Regulation of the $\text{E}_1\alpha$ subunit of PDC by phosphorylation and dephosphorylation. PDK1, pyruvate dehydrogenase kinase-1; PDP1, pyruvate dehydrogenase phosphatase-1. (C) Regulation of the $\text{E}_1\alpha$ subunit of PDC by acetylation/deacetylation. ACAT1, acetyl CoA acetyltransferase-1; SIRT3, NAD $^+$ -dependent deacetylase sirtuin 3. (D) Changes in the regulation and activity of PDC in cancer. LDH, lactate dehydrogenase; MCT4, monocarboxylate transporter-4 (also known as SLC16A3).

Fig. 2. Regulation of succinate dehydrogenase complex in mitochondria and its relevance to cancer

(A) Reaction mediated by succinate dehydrogenase complex (SDH). (B) Regulation of SDH by post-translational modification involving acetylation and deacetylation. ACAT1, acetyl CoA acetyltransferase-1; SIRT3, NAD⁺-dependent deacetylase sirtuin 3. (C) Regulation of SDH-B subunit of SDH by IRP1 at the level of translation. SIRT3, NAD⁺-dependent deacetylase sirtuin 3; IRP1, iron-regulatory protein-1; IRE, iron-responsive element; ROS, reactive oxygen species. (D) Changes in the regulation of SDH in cancer.

Fig. 3. Generation and release of lactic acid and the role of extracellular lactate in cancer cells
 Glucose is taken up by cancer cells at increased rate due to upregulation of the glucose transporters GLUT1 and SGLT1. As the oxidation of pyruvate is compromised in cancer cells due to mitochondrial dysfunction, this glycolytic end product gets converted to lactic acid. MCT4 facilitates the release of lactic acid from cancer cells in a H⁺-coupled manner. Extracellular lactate serves as an agonist for GPR81 expressed in the plasma membrane of cancer cells to decrease intracellular cAMP levels and also to increase intracellular Ca²⁺, which in turn promotes cell proliferation, angiogenesis, and chemoresistance.

3-Hydroxy-3-methylglutaryl CoA Synthase 2 (HMGCS2)

Fig. 4. Regulation of 3-hydroxy-3-methylglutaryl CoA synthase-2 (HMGCS2) and its relevance to cancer

(A) Reaction mediated by 3-hydroxy-3-methyl glutaryl CoA synthase-2. (B) Changes in the regulation of HMGCS2 in cancer. Overexpression of the oncogene c-Myc in cancer cells leads to the formation of Myc-Miz1 repression complex, which prevents recruitment of co-activators to the promoter of HMGCS2 gene, thus preventing transcription. Downregulation of SIRT3 in cancer leads to accumulation of HMGCS2 in acetylated form, which is a less active than the deacetylated form.

Fig. 5. Role of GPR109A in cancer prevention

In colon, butyrate serves as the agonist for GPR109A, which is expressed on the lumen-facing apical membrane of epithelial cells. Activation of the receptor in these cells provides protection against colitis and colon cancer. Butyrate enters colonocytes via multiple transport systems. Once inside the cell, butyrate inhibits HDACs and also gets converted into β -hydroxybutyrate (β -HB), which leaves the cell to enter portal circulation and then systemic circulation, most likely via monocarboxylate transporter MCT1 (SLC16A1). The levels of β -HB in systemic circulation are also elevated under caloric restriction conditions or by ketogenic diet. β -HB serves as the agonist for GPR109A that is expressed in the antigen-presenting cells (APCs) present in the colonic lamina propria, which leads to a tumor-suppressive condition in the colon. β -HB also activates GPR109A on cancer cells to increase apoptosis and decrease survival. HDAC, histone deacetylase; β -HB, β -hydroxybutyrate; APC, antigen-presenting cell; Tregs, regulatory T cells; IL-10, interleukin-10.

Table 1

Metabolite receptors and their endogenous and pharmacological agonists [67, 69, 74–76, 94, 115]

Receptor	Other names	Endogenous Agonists	Pharmacological Agonists
GPR 81	HCAR1	Lactate	3, 5-Dihydroxybenzoic acid 3-Cl-5-hydroxybenzoic acid 3-F-5-hydroxybenzoic acid Dichloroacetate Takeda Compound 2
GPR91	SUCNR1	Succinate	cis-Epoxy succinate cis-1,2-Cyclopropane dicarboxylate
GPR109A	HCAR2 NIACR1 PUMA-G HM74A	β -Hydroxybutyrate Butyrate	Niacin Acifran Monomethylfumarate SCH900271 MK1903 MK6892 GSK256073