Journal of Cancer Biology

Cornell et al., J Cancer Biol.
2024;5(2):65-75.

Review Article

Proton sensing GPCR's: The missing link to Warburg's oncogenic legacy?

Jessica Cornell[1], Samantha Rea[1], Leif R Neitzel[2,3], Charles H. Williams[2,3,*], Charles C. Hong[2,3,*]

[1]Department of Medicine, University of Maryland School of Medicine, Baltimore, MD, USA

[2]Department of Medicine, Michigan State University College of Human Medicine, East Lansing, MI, USA

[3]Henry Ford Health + Michigan State Health Sciences, Detroit, MI, USA

*Author for correspondence: Email: will4277@msu.edu, hongchar@msu.edu

Received date: July 26, 2024
Accepted date: October 21, 2024

Abstract

A century after Otto Warburg's seminal discovery of aerobic glycolysis in cancer cells, a phenomenon dubbed the "Warburg effect", the mechanistic links between this metabolic rewiring and tumorigenesis remain elusive. Warburg postulated that this enhanced glucose fermentation to lactate, even in the presence of oxygen, stemmed from an "irreversible respiratory injury" intrinsic to cancer cells. While oxidative phosphorylation yields higher ATP, the Warburg effect paradoxically persists, suggesting that the excess lactate and acid production are worth the deficit. Since Warburg's discovery, it has been demonstrated that the acidic tumor microenvironment activates a myriad of pro-oncogenic phenotypes ranging from therapeutic resistance to immune escape. Here we propose that proton-sensing G-protein-coupled receptors (GPCRs) act as crucial heirs to Warburg's findings by transducing the acid signal from elevated glycolytic lactate into pro-oncogenic signals.

The increased lactate production characteristic of the Warburg effect causes extracellular acidification. This acidic tumor microenvironment can activate proton-sensing GPCRs like GPR68, a proton-sensing receptor shown to stimulate proliferation, migration, and survival pathways in cancer cells. Such pH sensing is accomplished through protonation of key residues such as histidine, which causes a conformational change to activate various downstream signaling cascades including the MAPK, PI3K/Akt, Rho, and β-arrestin pathways implicated in tumor progression and therapeutic resistance. By coupling Warburg's "respiratory injury" to potent mitogenic signaling, proton-sensing GPCRs like GPR68 may unveil a longstanding mystery – why forgo efficient ATP generation? As heirs to Warburg's iconic metabolic observations, these proton sensors could represent novel therapeutic targets to disrupt the synergy between the Warburg effect and oncogenic signaling.

Introduction

A hundred years have passed since Otto Warburg first described aerobic glycolysis which came to be known as the Warburg effect, a phenomenon in which cancer cells preferentially use glycolysis to generate energy, even in the presence of oxygen [1]. This process, earning Warburg the Nobel Prize in 1931, contrasted with non-cancerous cells which primarily use oxidative phosphorylation to generate energy. Warburg observed that cancer cells had significantly higher levels of lactate production and lower levels of oxygen consumption compared to normal cells [2]. Based on his findings, Warburg proposed that the increased glucose uptake and lactate production in cancer cells was due to a defect in their ability to perform oxidative phosphorylation; thus, aerobic glycolysis was a compensatory mechanism to maintain ATP production. However, this interpretation of the data has been challenged since the early 1950s [3]. Notably many studies have shown that oxidative phosphorylation occurs in cells with normal mitochondrial ability and intact cytochromes [4-8]. The Warburg effect as it is now understood is the conversion of glucose to lactate in the presence of oxygen and functioning mitochondria, typically due to changes in regulation of metabolism as opposed to damage to respiration.

Despite the numerous studies into the Warburg effect, there is still a lack of clarity about why proliferating tumor cells engage in aerobic glycolysis when its counterpart, oxidative phosphorylation, produces a higher yield of ATP. With the inefficient production of ATP production by aerobic glycolysis that many cancers favor, this begs the question "Why do cancer cells prefer to run an energy deficit to metabolize glucose?" An emerging hypothesis considers the Warburg effect in the opposite direction: instead of primarily viewing lactate as a byproduct of metabolism to fuel proliferation, lactate and protons are viewed as the primary product, while ATP and energy is a byproduct. The acidification of the tumor microenvironment (TME) due to the increased production of lactate and protons has shown to promote chemoresistance, drive cancer progression, and increase poor patient outcomes [9,10]. In turn, this allows for the stimulation of cytoprotective signaling pathways through acid-sensing G-protein coupled receptors (GPRC) like GPR68. Here, we discuss proton-sensing GPCRs as an answer to cancer's preference in altering their metabolism towards the Warburg effect.

The Warburg Paradox: Why Forgo Efficient ATP Production?

Understanding the contrast between oxidative phosphorylation and aerobic glycolysis is crucial to appreciate the paradoxical nature of the Warburg effect. Oxidative phosphorylation, the primary ATP generator in normal cells, is an extremely efficient process occurring in the mitochondria. It couples electron transfer through the respiratory chain complexes to pumping of protons across the inner mitochondrial membrane, establishing an electrochemical gradient that powers ATP synthase to produce approximately 36 ATP molecules per glucose molecule [1-3].

In stark contrast, aerobic glycolysis, the hallmark of the Warburg effect, is a cytosolic process that rapidly metabolizes glucose to lactate while netting only 2 ATP molecules per glucose. This dramatic difference in ATP yield makes the prevalence of aerobic glycolysis in proliferating cancer cells paradoxical from a bioenergetic perspective. Despite this inefficiency, the Warburg effect persists, suggesting it confers other advantages beyond maximizing ATP production.

The glycolytic switch is facilitated by upregulation of key enzymes like pyruvate kinase M2 (PKM2) [3-6] which catalyzes the final ATP-generating step, and hexokinase 2 (HK2) [3-7] which phosphorylates glucose for entry into the pathway. Moreover, transcription factors like HIF-1α [8-10] induce expression of other glycolytic enzymes and glucose transporters like GLUT1 [11-19], further amplifying this metabolic rewiring. While portrayed as a desperate attempt to sustain growth, the Warburg effect is an active process driven by complex regulatory mechanisms.

Although aerobic glycolysis is remarkably inefficient compared to oxidative phosphorylation in ATP yield per glucose, cancer cells are not universally glycolytic [20]. A fraction of their metabolism can flux through the TCA cycle, suggesting oxidative phosphorylation remains active to a limited degree. Nonetheless, a profound question lingers – if not solely for ATP production, what advantage does the "inefficient" Warburg effect provide proliferating tumor cells to persist as such a widespread phenomenon? Lactate and acid which are often portrayed as bystanders of aerobic glycolysis, contribute to cancer progression by fostering malignant clonal selection, metastasis, and immune escape [21-28]. Proton-sensing GPCRs may unveil this paradox by coupling lactate acidification to oncogenic signaling pathways.

Lactic Acidosis: The Purposeful Cost of the Warburg Effect?

The Warburg effect's bioenergetic inefficiency, trading 36 potential ATP molecules from oxidative phosphorylation for a mere 2 from glycolysis per glucose, has long perplexed researchers. If ATP generation is not the principal aim, what selective advantage could justify cancer cells willingly operating at an energy deficit? An emerging hypothesis reframes the Warburg paradox by proposing that lactate and protons, rather than ATP, are the primary intended products, with energy being a fortuitous secondary gain.

Supporting this idea, studies suggest ATP levels may not be limiting for cancer cell proliferation [29]. In fact, excess ATP could impair mitochondrial function, while "futile cycles" that consume ATP paradoxically promote tumor growth [30]. PTEN-deficient cancer cells upregulate ATP-wasting enzymes, and elevated ATP correlates with impaired tumor progression [31]. Notably, insufficient ATP synthase activity can limit NAD+ regeneration, a crucial cofactor [29]. Thus, pathways that consume ATP may indirectly boost proliferation by maintaining NAD+ pools.

Furthermore, with the production of lactate and protons from aerobic metabolism, tumor cells can be selected towards higher proton transport channels to reduce intracellular toxicity [32,33]. Lactate and H+ cotransporters like monocarboxylate transporters MCT1 and MCT4, show increased expression levels in tumor cells [34]. Additionally, the transmembrane protein induced by hypoxia inducible factor 1 subunit alpha (HIF1α), carbonic anhydrase isoform 9 (CA9) and 12 (CA12), are overexpressed in tumor cells and are responsible for catalyzing the reversible hydration of carbon dioxide, producing H+ [35,36]. CA9 has been shown to enhance MCT1 and MCT4, and lactate has been shown to stabilize HIF1a expression, further acidifying the extracellular TME [37]. Proton exchangers like vacuolar H^+-ATPase (V-ATPase) and Na^+/H^+ antiporter 1 (NHE1) can also contribute to extracellular acidification when activated [38-40] **(Figure 1)**. The Acidic TME has been shown to drive tumor malignancy including proliferation, invasion, metastasis, immune evasion, and cell death avoidance [21,23-25,27].

From this perspective, the Warburg effect represents not simply an upregulation of glycolysis at oxidative phosphorylation's expense. Instead, it reflects a metabolic reprogramming where pyruvate is diverted away from the TCA cycle and channeled towards lactate fermentation. While counterintuitive from an energetic view, this metabolic shift may serve a distinct purpose – generating and exporting lactate and its associated acidity as intended products to produce a proton-rich tumor microenvironment.

Proton-Sensing GPCRs: Warburg's Metabolic Signal Transducers

The vertebrate repertoire to detect the acidity characteristic of the tumor microenvironment is surprisingly limited. Standing out as promising candidates are the proton-sensing G-protein-coupled receptors (GPCRs), a specialized family exquisitely tuned to transduce extracellular acidification into intracellular signals. Broadly, GPCRs are the largest membrane protein family, consisting of seven transmembrane α-helices, an extracellular N-terminus, and an intracellular C-terminus. GPCRs mediate cellular responses to various stimuli including neurotransmitters and hormones [41].

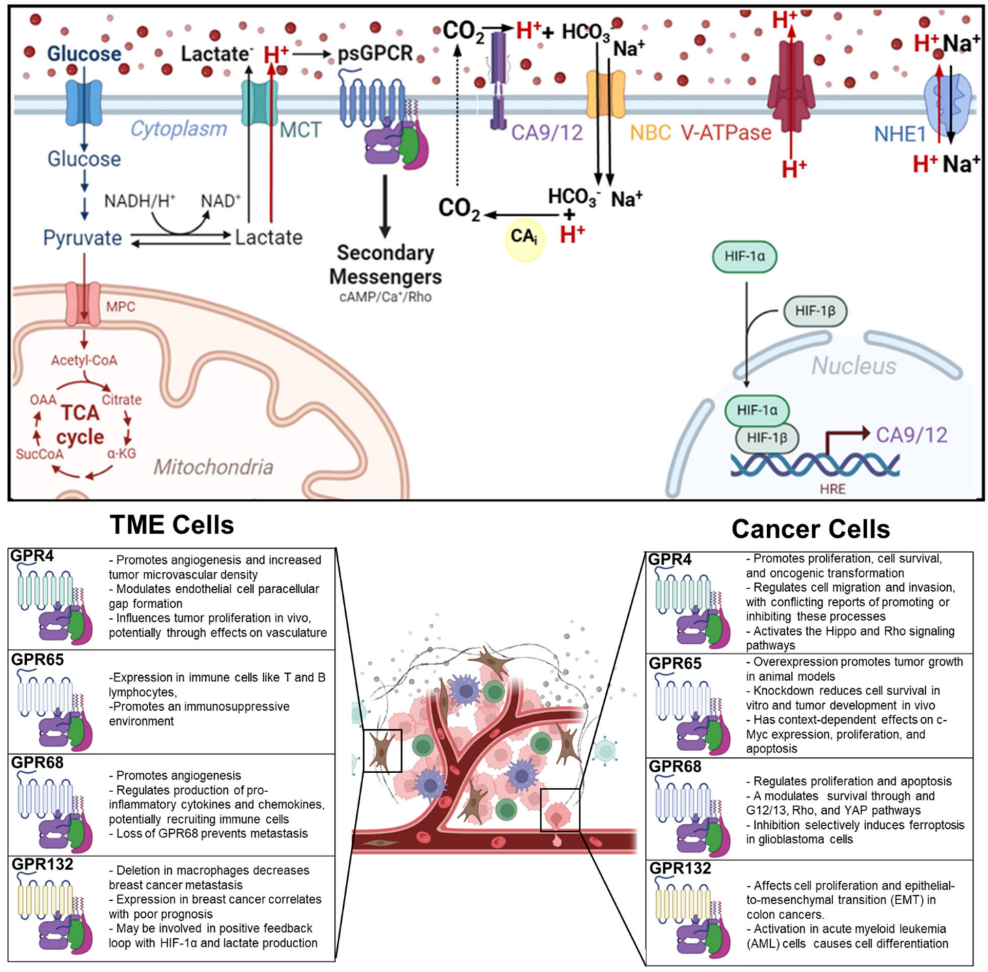

Figure 1. Proton-sensing GPCRs in the Tumor Microenvironment and Cancer Progression (Top) The metabolic and molecular processes leading to extracellular acidification in cancer cells. These processes include glucose metabolism through aerobic glycolysis (the Warburg effect), lactate production and export via MCT transporters, CO_2 hydration facilitated by carbonic anhydrases (CA9/12), proton extrusion through transporters such as V-ATPase and NHE1, and the activation of proton-sensing GPCRs (psGPCRs) by extracellular H^+. The activation of HIF-1α induces the expression of CA9/12. **(Bottom left)** The functions of four proton-sensing GPCRs (GPR4, GPR65, GPR68, and GPR132) within TME cells. **(Bottom Right)** The functions of four proton-sensing GPCRs (GPR4, GPR65, GPR68, and GPR132) within cancer cells responding to the autocrine acidification caused by the Warburg effect.

Upon proton activation, proton-sensing GPCRs initiate downstream signaling cascades like MAPK/ERK, PIP/PLC, and Rho – pathways intimately linked to driving hallmarks of malignancy including proliferative signaling, resisting cell death, activating invasion and metastasis [42,43]. GPR68 which is the prototypical proton sensing GPCR is inactive at physiologic pH 7.4 but becomes fully activated as the surrounding milieu acidifies to pH 6.4 – conditions often found in the glycolytic microenvironments of solid tumors [44]. Proton-sensing GPCRs' ability to directly couple the acidic byproduct of aerobic glycolysis to potent oncogenic signaling implicates proton-sensing GPCRs as crucial signal transducers underlying Warburg's metabolic observations **(Figure 2)**.

Representing nearly a third of all pharmaceutical targets, GPCRs are an established and attractive class for therapeutic development [45,46]. Arora, *et al.* combined transcriptomic datasets with patient-survival data to further understand the vast network of GPCR signaling pathway in tumor cells and give insight into additional therapeutic targets [47]. Notably, there are a small handful of the GPCR targeting therapeutics for cancer indications **(Table 1)**. Targeting specific proton-sensors could disrupt the synergy between Warburg's metabolic derangement and downstream oncogenic signaling. Below, we will highlight the numerous studies demonstrating the multifaceted roles of proton sensing GPCRs in promoting both cancer cell-autonomous and non-autonomous mechanisms of tumor progression.

GPR4 and cancer

GPR4 is expressed in a variety of tissues and cell types, ranging from neurons and immune cells to vascular cells. In skin cancers, GPR4 is expressed in compound nevus cell nevi, squamous cell carcinoma, basal cell carcinoma and malignant melanoma, with malignant melanoma expression being highest among skin cancers

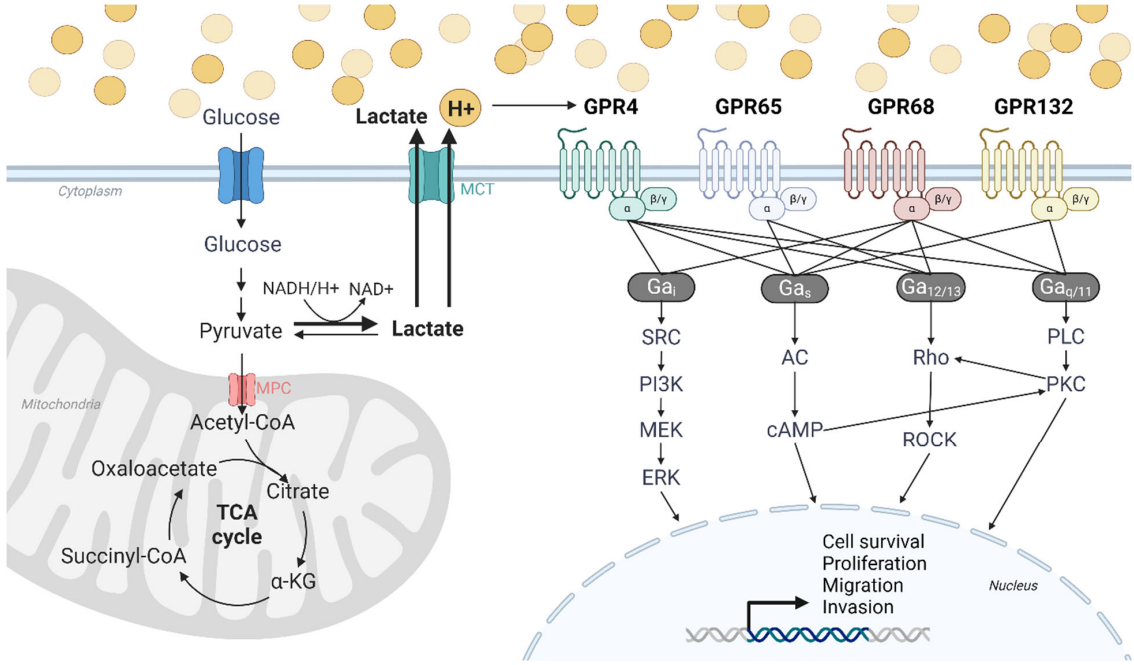

Figure 2. Multiple Proton-sensing GPCR Signal through G proteins in Response to the Tumor Microenvironment. The metabolic and molecular processes leading to extracellular acidification in cancer cells. These processes include glucose metabolism through aerobic glycolysis (the Warburg effect), lactate production and export via MCT transporters, and the activation of proton-sensing GPCRs (psGPCRs) by extracellular H^+. The functions of four proton-sensing GPCRs (GPR4, GPR65, GPR68, and GPR132) within cancer cells are mediated through a diversity of Ga proteins.

Table 1. Table of GPCRs with clinically verified effects on cancer. Molecules in Bold are FDA approved for cancer treatment. Abbreviations: CXCR4, C-X-C motif chemokine receptor 4; D1R, dopamine receptor 1; GnRHR, gonadotropin releasing hormone receptor; LH, luteinizing hormone; FSH, Follicle-stimulating hormone; SMO, smoothened; SSTR, Somatostatin receptor. *Prolactinoma is a non-cancerous tumor.

GPCR	Approved Indication	Function	Agonist	Antagonist	Citations
CXCR4	Multiple myeloma, Non-Hodgkin lymphoma, T-cell Leukemia	Promotes cell migration, invasion, and metastasis	ATI-2341	BKT140 Balixafortide Mavorixafor **Plerixafor**	[114-116]
D1R	Prolactinoma*	Regulates cell proliferation, growth, and apoptosis	SKF 81297 Fenoldopam	SCH23390 SCH39166	[117]
GnRHR	Prostate cancer, Breast cancer	Regulates secretion of LH and FSH hormones	Buserelin **Goserelin** Leuprorelin **Triptorelin**	**Degarelix** Linzagolix **Relugolix**	[118]
SMO	Basal cell carcinoma	Regulate cell differentiation, growth, and invasion	Clobetasol Halcinonide	**Sonidegib** **Vismodegib**	[119,120]
SSTR	Gastroenteropancreatic neuroendocrine tumors, Carcinoid tumors	Regulate hormonal secretion, cell proliferation and apoptosis	Lanreotide **Lutathera** Octreotide	Pasireotide	[121-123]

[48]. GPR4 expression promotes progression in colorectal cancer cells and ovarian cancer and causes oncogenic transformation when expressed in 3T3 cells [49-51]. In colon cancer cells, GPR4 expression drives Hippo pathway activation with loss of GPR4 decreasing both HIPPO responsive genes, phosphorylation, and cell survival [51].

Besides its role in cell survival and proliferation, GPR4 has been implicated in regulating tumor cell motility with conflicting roles. In both melanoma cells and prostate cancer cells, over-expressing GPR4 strongly inhibited migration and invasion *in vitro* and reduced pulmonary metastasis [52]. However, GPR4 over-expressing SK-Mel-28 cells showed increased migration in impedance-based electrical wounding and migration assay. Yet, in the same publication, the same cells only showed increased migration at pH 7.5 in Boyden chamber experiments, but not at pH 6.5 [53]. While these potentially conflicting findings could result from contextual differences in assays, multiple studies provide strong evidence of GPR4 promoting activation Rho. RhoA activation leads to the formation of stress fibers and focal adhesions, which are essential for cell adhesion and migration. Stress fibers are composed of thick bundles of actin filaments that provide structural support for the cell. Focal adhesions are complex protein structures that anchor the cell to the extracellular matrix and provide a mechanical link between the actin cytoskeleton and the extracellular environment [54,55]. Similarly, RhoA activation also promotes the formation of actomyosin contractile forces, which are important for cell movement. Actomyosin contractile forces are generated by the interaction between actin filaments and myosin motor proteins. Notably, melanoma cells also have polarized distribution of extracellular pH, and even more acidic domains created by NHE1 at focal adhesions [56,57].

Beyond cell autonomous roles, GPR4 activation has also been implicated in cancer progression by influencing non cancer cells as well. In two separate orthotopic models, angiogenesis and tumor proliferation were both decreased in mice lacking GPR4 [58]. Consistent with this finding, GPR4 was found to promote angiogenesis in head and neck cancer, and high levels of GPR4 expression are correlated with increased microvascular density in epithelial Ovarian carcinoma [59,60]. This ability to modulate tumor vasculature is thought to be through modulating VEGFR2 receptor expression in the local endothelial cells [58]. Notably, the role of GPR4 in endothelial cells also extends to the formation of the paracellular gap [61]. Taken together, GPR4 regulates migration, however, further study is needed to fully understand how and where endogenous GPR4 is activated in cells, how that influences tumor cell behavior, and how these findings translate to a tumor *in vivo*.

GPR65 (TDAG8) and cancer

GPR65, also known as TDAG8, is a proton-sensing G-protein-coupled receptor that plays a crucial role in various cellular processes. Its expression has been detected in immune cells, including T and B lymphocytes, as well as in lymphoid tissues, lungs, and small intestines [62]. In cancer, GPR65 is overexpressed in several tumor types, including squamous cell carcinoma, epidermal malignant melanoma, and dermal portions of nevus cell nevi [51]. GPR65 has also been detected in kidney, ovarian, breast, and colon cancers [48,63-65]. Overexpression of GPR65 in Lewis lung carcinoma (LLC) cells has been shown to promote tumor growth in animal models [66]. Similarly, knock down of GPR65 in NCI-H460 human non-small cell lung cancer cells reduced cell survival *in vitro* and decreased tumor development *in vivo* [67]. These findings suggest GPR65 may contribute to tumor progression by enhancing cell survival and proliferation [66]. However, acidic activation of the GPR65 receptor decreases the expression of c-Myc in human lymphoma cells, which reduces proliferation and tumorigenesis [67]. This indicates a complex, context-dependent role of GPR65 in different cancer types and cellular environments.

In addition to its role in tumor cells, GPR65 significantly impacts the tumor microenvironment (TME). The TME, which consists of various cell types, including immune cells, fibroblasts, and endothelial cells, is crucial for tumor progression and metastasis. GPR65 is known to modulate immune cell function within the TME. Its expression in immune cells, such as T and B lymphocytes, and its regulation of the immune microenvironment are critical for cancer progression [67,68]. The receptor's activity has been linked to both the activation and inhibition of apoptosis in different cellular contexts, suggesting that it might help create an immune-suppressive environment that favors tumor growth [69]. Moreover, GPR65 has been identified as a potential immune checkpoint, regulating the immune response within the TME [67]. By influencing the immune cells' behavior and potentially promoting an acidic TME, GPR65 contributes to a milieu that supports tumor survival and growth. Thus, GPR65's dual role in both tumor cells and the TME highlights its importance as a target for cancer therapy, with the potential to disrupt tumor-promoting signals and enhance anti-tumor immunity [70,71]. Therefore, it is thought that GPR65 might have context dependent effects in cancer progression.

GPR68 and cancer

GPR68, also known as OGR1, is expressed in a variety of tissues and cell types, including the spleen, testis, small intestine, peripheral blood leukocytes (PBL), placenta, kidney, brain, lung, vasculature, heart, and pancreas [72-76]. In a survey of skin cancers, GPR68 was rarely seen in squamous cell carcinoma but like GPR4, and GPR65 was found in Nevus cell nevi and malignant melanoma [35]. GPR68 expression is expressed in a number of cancers including pancreatic ductal adenocarcinoma [77,78], lung cancer, osteosarcoma MG-63 cells [79], medulloblastoma [80,81], Merkel cell carcinoma (MCC) [82] and glioblastoma (GBM). Notably, GPR68 is upregulated in both breast cancer and head and neck cancer when compared to control tissue [83,84]. Furthermore, in both these cancers increased GPR68 was found to correlate to worse outcomes [83,84].

In cell autonomous roles, GPR68 is required for pH-dependent regulation of proliferation and apoptosis through the G protein α subunit 12/13 (G12/13) in -Rho GTPases-YAP pathway [85]. Consistent with an increased proliferation in response to acidic media SKOV-3 (ovarian) and ECC1 (endometrium), both lines express GPR68. Despite the high degree of molecular heterogeneity, a wide array of glioblastoma cell lines have demonstrated sensitivity to GPR68 inhibition. Williams *et al.* demonstrated *in vitro* and Neitzel *et al.* demonstrated *in vivo* that GPR68 proteins in glioblastoma sense acid produced in an autocrine manner. They found that inhibiting GPR68 with specific small molecule inhibitors blocked this sensing mechanism, which in turn induced ferroptosis—a regulated, iron-dependent form of cell death—in GBM cells. This process involved the upregulation of ATF4 and its target CHAC1, leading to glutathione depletion and subsequent lipid peroxidation. Notably, the inhibition of GPR68 selectively triggered ferroptosis in GBM cells while leaving non-cancerous cells unaffected [86,87].

GPR68 has also been shown to play a role in cancer progression by modulating the tumor microenvironment. For example, GPR68 has been shown to promote angiogenesis in tumors [88,89], which is essential for tumor growth and metastasis GPR68 has also been shown to regulate the production of pro-inflammatory cytokines and chemokines, which can recruit immune cells to the tumor and promote inflammation. In summary, GPR68 plays both cell autonomous and non-cell autonomous roles in cancer progression, including regulating cell proliferation, migration, and invasion, as well as modulating the tumor microenvironment and promoting angiogenesis and inflammation.

GPR132 and cancer

Due to its stabilizing effects on HIF-1α and ability to influence macrophage signaling, lactate may also be proposed as a primary product of the Warburg effect. Lactate is known to activate a G-coupled protein receptor called GPR132, also known as G2A, in macrophages. GPR132 deletion in a mouse model of breast cancer has been shown to inhibit tumor growth, suggesting that GPR132 may play a role in promoting breast cancer cell proliferation and survival [65]. In human colon cancer cells, silencing GPR132 affects cell proliferation and epithelial-to-mesenchymal transition (EMT), indicating GPR132 might be involved in maintaining the cancerous characteristics of colon cancer cells [90].

In acute myeloid leukemia (AML) cells, GPR132 activation can lead to cell differentiation. The activation of GPR132 in AML has therapeutic potential, as evidenced by the use of specific agonists to probe its role in treating the disease [91]. GPR132 also plays a role in the interaction between cancer cells and tumor-associated macrophages. Cancer cell-derived lactate activates macrophage GPR132, which in turn promotes cancer metastasis, indicating GPR132 facilitates a pro-tumoral environment [92]. Deletion of GPR132 in macrophages has been shown to decrease metastasis of breast cancer. In addition, GPR132 expression in breast cancers is noted to be correlated with poor prognosis. Furthermore, HIF-1α can further stimulate lactate production, by signaling through genes like mucin MUC1, thus potentially providing positive feedback for lactate production/to continually activate HIF-1α.

Small Molecule Modulators of Proton Sensing GPCRs

G protein-coupled receptors (GPCRs) represent a large proportion of pharmacological drug targets [45,93]. In the case of proton-sensing GPCRs, various small molecule modulators have been developed, offering opportunities for therapeutic intervention [94,95].

For GPR4, while no agonists are currently known, two antagonists have been identified: NE 52-QQ57 [96] and GPR4 Antagonist C39c [97], demonstrating the receptor's amenability to chemical modulation.

In contrast, GPR65 has several characterized pharmacological tools. BTB09089 [98] and ZINC13684400 [94] function as allosteric agonists, while ZINC62678696 acts as an allosteric inhibitor of BTB09089 activation [94]. Although these molecules have primarily been used to study GPR65's role in inflammation, Pathios Therapeutics recently presented findings at AACR highlighting a GPR65 antagonist that characterized the receptor's critical role in mediating immunosuppressive signaling in tumor-associated macrophages [99].

For GPR68, numerous agonists have emerged, including a family of 3-5 disubstituted isoxazoles initially described [73], followed by the positive allosteric modulator ogerin and MS48107 [100]. Notably, the use of lorazepam, a benzodiazepine with off-target GPR68 agonist activity, correlated with worse outcomes in pancreatic cancer patients [101]. Regarding antagonists, ogremorphin is commercially available [86], while Takeda has distributed its GPR68 inhibitor GPR68-I, and Certa Therapeutics is investigating FT011, a GPR68 antagonist, for renal fibrosis [102].

Finally, for GPR132, NOX-6-7 and ONC212 (a fluorinated analog of ONC201) function as agonists [103,104], while NOX-6-18 acts as an antagonist [103]. This target is being developed for treatment of diabetes and attenuation of weight gain. The anti-cancer compound ONC212 has been shown to activate GPR132 in tumor cells, and silencing GPR132 affects the cytotoxicity of ONC212, suggesting GPR132 is a critical target for this compound's anti-cancer effects [104].

This growing arsenal of small molecule modulators targeting proton-sensing GPCRs holds promise for therapeutic applications by modulating the signaling pathways downstream of these pH-sensing receptors.

Challenges and Considerations in Using Small Molecule Modulators of Proton Sensing GPCRs

While small molecule modulators of proton-sensing GPCRs offer promising therapeutic avenues, there are notable challenges associated with their specificity and potential toxicities. First off target effects must be considered, many GPCR modulators have off target effects on other GPCRs, leading to unintended activation or inhibition of signaling pathways that are crucial for normal physiological functions. Clozapine, an antipsychotic drug is primarily a serotonin 5-HT2A receptor antagonist but also binds to dopamine receptors, adrenergic receptors, and muscarinic acetylcholine receptors, spanning multiple GPCR families. Similarly, Propranolol is primarily a β-adrenergic receptor antagonist, but it also interacts with 5-HT1A receptors, affecting serotonin pathways, demonstrating its activity beyond adrenergic GPCRs.

Off-target effects are a significant concern in drug development, particularly when targeting receptors within the same family. The cannabinoid receptors, specifically CB1 and CB2, exemplify this issue. Nabilone, a synthetic cannabinoid approved by the FDA, exhibits a modestly higher affinity for the CB1 receptor compared to CB2, with a ratio of approximately 2:1 [105]. This slight difference in affinity can lead to unintended interactions, potentially resulting in off-target effects that complicate therapeutic outcomes [106,107].

In the context of receptor antagonism, the knockout phenotypes of the proton sensing G protein-coupled receptors GPR4, GPR65, GPR68, and GPR132 provide critical insights into the physiological roles of these receptors and their associated side effects. For instance, the knockout of GPR4 in mice has been linked to severe vascular abnormalities, including spontaneous hemorrhages and defective vascular smooth muscle cell coverage, indicating a crucial role in vascular development and permeability [61]. This suggests that antagonism of GPR4 could lead to significant vascular instability and hemorrhagic events. Similarly, GPR65 knockout mice exhibit defects in immune system development, which raises concerns that small molecule antagonists targeting this receptor could disrupt

normal immune function, despite these mice showing resistance to various immune disorders [108-110].

The GPR68 receptor has been implicated in tooth enamel formation and osteoclastogenesis, with its knockout in mice revealing no severe health concerns in adults, although it does play a role in dental health [111]. In contrast, GPR132 knockout mice appear phenotypically normal but develop late-onset lymphoproliferative autoimmune syndrome, suggesting that prolonged antagonism of this receptor may have deleterious effects [112]. These findings underscore the necessity for drug development projects to carefully consider the potential liabilities associated with receptor antagonism, as addressing these limitations could enhance therapeutic efficacy while minimizing adverse effects. Notably allosteric modulators are an important tool in this respect as they might mitigate some of the known on-target toxicities that may exist [113].

In summary, the intricate dynamics of small molecule and receptors, particularly within the GPCR families, necessitate a thorough understanding of the physiological roles of these receptors to mitigate undesirable effects during drug development. Future research should focus on refining drug design to optimize therapeutic outcomes while minimizing risks associated with off-target effects, or negative on target effects.

Conclusion

A century after Otto Warburg's groundbreaking observations, we are still uncovering deeper significance behind the Warburg effect and aerobic glycolysis in cancer cells. While initially perplexing in its bioenergetic inefficiency, this metabolic rewiring may represent an adaptive strategy to purposefully generate lactate and protonic acid as key drivers of malignancy.

As the heirs to Warburg's metabolic findings, proton-sensing GPCRs like GPR68, GPR4, GPR65, and GPR132 transduce the acidic signal from elevated glycolytic lactate into potent oncogenic signaling cascades. Through activation of proliferative, migratory, and pro-survival pathways like MAPK, PI3K/Akt, Rho, and β-arrestin, these pH sensors can couple Warburg's "respiratory injury" to tangible hallmarks of cancer progression.

Moreover, acid-sensing GPCRs exert influence beyond cancer cell-autonomous effects. Their activation modulates the tumor microenvironment, promoting angiogenesis, inflammation, and immune escape – processes crucial for continued tumor growth and metastasis. The impacts of lactate and acidosis on vascular permeability, immune suppression, and therapy resistance underscore the relevance of proton sensing in cancer's pathogenesis.

As our understanding of these key pH transducers advances, proton-sensing GPCRs emerge as intriguing therapeutic targets with the potential to disrupt the synergistic relationship between Warburg's archetypal metabolic derangement and core oncogenic mechanisms. Targeting this family could blunt cancer's ability to exploit acidosis for its proliferative advantage. In this light, acid-sensing GPCRs represent an underexplored frontier – the long-awaited molecular heirs poised to elucidate and antagonize Warburg's paradoxical metabolic legacy.

Competing Interests

CHW and CCH are inventors on an issued patent related to this manuscript. JC, SR, CHW, and CCH are inventors on a patent application related to this manuscript.

Funding

This work was funded by NIGMS R01GM118557 and NIH NHLBI R01HL167192A1 to CCH, LRN was supported by T32 AR007592-26.

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