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Review Article

Revisiting the Warburg Effect: Linking Hydrogen Sulfide to the Central Metabolic Network in Cancer

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Abstract

The Warburg effect is a metabolic process in which cancer cells prefer glycolysis over aerobic respiration even in the presence of oxygen. This metabolic shift allows cancer cells to use glycolytic intermediates for synthesizing DNA, RNA, and proteins, enabling rapid proliferation. Among other known regulators of cancer metabolism, hydrogen sulfide (H_2S), a gasotransmitter produced endogenously inside the cell, has emerged as a key player. H_2S mediates protein sulfhydration, a posttranslational modification resulting in changes of protein dynamics to modulate the activity of glycolytic enzymes, including pyruvate kinase M2 (PKM2), lactate dehydrogenase A, and glyceraldehyde-3-phosphate dehydrogenase. Sulfhydration of PKM2 at cysteine 326, in particular, promotes its transition from an active tetrameric state to a less active dimeric state, redirecting glucose metabolism to glycolysis and facilitating tumor growth. This review highlights the importance of H_2S-mediated sulfhydration in cancer metabolism and advocates for further exploration of this pathway as a target for anticancer drug development. A deeper understanding of the biochemical pathways underlying cancer metabolism will facilitate the design of highly selective and potent inhibitors, ultimately improving therapeutic outcomes for cancer patients.

Keywords: Glucose metabolism, hydrogen sulfide, pyruvate kinase M2, sulfhydration, Warburg effect

Introduction

Cancer metabolism refers to alterations in cellular metabolic pathways that enable cancer cells to acquire essential nutrients for building new biomass.[1] The metabolic rewiring in cancer cells has recently been recognized as a major hallmark of cancer.[2] Metabolic reprogramming is often driven by diverse signaling cues from the tumor microenvironment, which influence cancer cell metabolism by altering the expression and activity of key metabolic enzymes.[1] Glucose is the main energy source in cell metabolism and a key material for biosynthesis. In 1927, German physiologist Otto Warburg first observed that tumors consume significantly more glucose and rely preferentially

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on glycolysis, even in the presence of oxygen, compared to nonproliferating normal tissues.[3,4] This phenomenon is widely known as the Warburg effect. Although glycolysis is less efficient than oxidative phosphorylation (OXPHOS) regarding the production of adenosine triphosphate (ATP), glycolysis provides metabolic intermediates required for the synthesis of macromolecular biosynthesis, thus enabling cancer cells to proliferate faster under insufficient nutrient supply.[1] In this review, we provide a brief overview of recent developments connecting hydrogen sulfide (H_2S), a gasotransmitter, to this classical metabolic rewiring mechanism in cancer.

The Warburg Effect

The breakdown of carbohydrates, particularly glucose, is a major source of cellular energy. Glucose metabolism occurs through two main pathways: OXPHOS and anaerobic glycolysis.[5] Under aerobic conditions, most differentiated cells metabolize glucose through the tricarboxylic acid cycle and OXPHOS, generating 36 ATP per glucose. In contrast, glycolysis alone produces just 2 ATP per glucose.[5] However, in the absence of oxygen, normal differentiated cells undergo anaerobic glycolysis, redirecting pyruvate molecules to lactate production.[6] Unlike normal differentiated cells, most cancer cells preferentially rely on glycolysis for energy production, regardless of whether oxygen is present. Although glycolysis produces ATP more rapidly than OXPHOS, it is far less efficient in producing ATP per glucose consumed, and consequently, cancer cells have to consume a much higher amount of glucose to satisfy their energy demands.[2] This phenomenon, first discovered by Otto Warburg in 1927, is known as the Warburg effect.[3]

The discovery of the Warburg effect raised the question of why cancer cells rely on aerobic glycolysis, an inefficient process that produces over ten times less ATP per cycle compared to OXPHOS. Based on the underlying biochemical mechanisms, it has been proposed that the Warburg effect is a metabolic adaptation to meet the biosynthetic demands of cancer cells for rapid proliferation.[7] During glycolysis, cancer cells use glycolytic intermediates as carbon sources for nucleotide, fatty acid, and amino acid synthesis [Figure 1]. Two key glycolytic intermediates, glucose-6-phosphate and 3-phosphoglycerate, play central roles in this process for the *de novo* synthesis of nucleotides, amino acids, and lipids through the pentose phosphate pathway[8] and one-carbon metabolism,[9] respectively. Current evidence suggests that not only cancer cells but also normal proliferating cells prioritize glucose consumption to produce glycolytic intermediates that meet the biosynthetic demands of cell division.[5] To achieve this, they modulate glycolysis by slowing down its final step: the conversion of phosphoenolpyruvate (PEP) to pyruvate, catalyzed by pyruvate kinase (PK).[10]

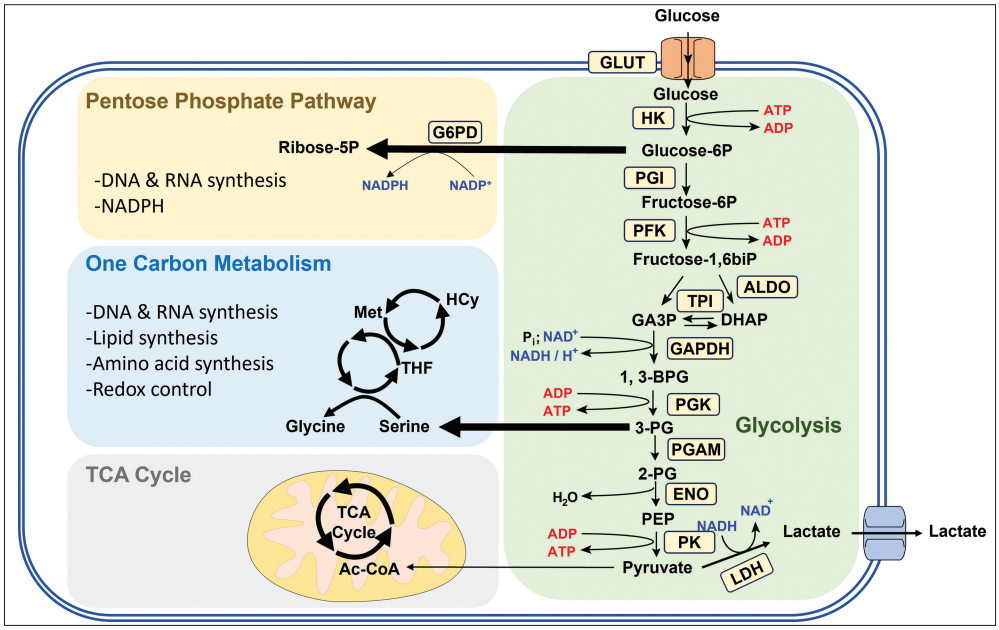

Figure 1: Reprogramming of glucose metabolism in cancer. Glycolysis plays a central role in cancer cell metabolism, not only as a source of energy but also by providing intermediates for biosynthesis and redox homeostasis. Cancer cells utilize glycolytic intermediates, such as Glucose-6P and 3-PG, to drive nucleotide, lipid, and amino acid synthesis through pathways like the pentose phosphate pathway and one-carbon metabolism. In addition, cancer cells often redirect pyruvate away from the tricarboxylic acid cycle in mitochondria, favoring lactate production to support their metabolic needs. 1,3-BPG: 1,3-Bisphosphoglycerate; 2-PG: 2-phosphoglycerate; 3-PG: 3-phosphoglycerate; Ac-CoA: Acetyl-CoA; ADP: adenosine diphosphate; ALDO: aldolase; ATP: adenosine triphosphate; DHAP: dihydroxyacetone phosphate; ENO: enolase; G6PD: glucose-6-phosphate dehydrogenase; GA3P: glyceraldehyde 3-phosphate; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; GDP: guanosine diphosphate; GLUT: glucose transporter; HCy: homocysteine; HK: hexokinase, LDH: lactate dehydrogenase; 4; Met: methionine; NAD+: nicotinamide adenine dinucleotide; NADP: nicotinamide adenine dinucleotide phosphate; PEP: phosphoenolpyruvate; PFK: phosphofructokinase; PGAM: phosphoglycerate mutase; PGI: phosphoglucoisomerase; PGK: phosphoglycerate kinase; PK: pyruvate kinase; THF: tetrahydrofolate; TPI: triosephosphate isomerase

Pyruvate Kinase M2

PK is a rate-limiting enzyme of glycolysis that catalyzes phosphoryl transfer from PEP to adenosine diphosphate to produce pyruvate and ATP.[11] In mammals, two genes encode four mammalian PK isoforms (PKM1, PKM2, PKR, and PKL). The *PKLR* gene encodes PKL and PKR, which are expressed in the liver and erythrocytes. The *PKM* gene encodes PKM1 and PKM2 through alternative splicing. PKM1 is expressed in many normal differentiated tissues, while PKM2 is expressed in proliferating cells, including in all types of cancer cells and tumors tested so far.[12] Despite their sequence similarity, PKM1 and PKM2 have distinct catalytic properties. PKM1 is constitutively organized as a high-activity tetramer, while PKM2 exists in multiple oligomeric states: a highly active tetramer, less active dimer, or monomer.[13] The tetrameric state of PKM2 is endogenously activated by fructose-1,6-bisphosphate (FBP),[14] serine,[15] and succinyl-5-aminoimidazole-4-carboxamide ribose-5'-phosphate (SAICAR).[16] In contrast to other tetrameric PK isoforms, PKM2 can be regulated by multiple factors that promote the release of FBP, thereby reducing enzyme activity through dissociation from tetrameric to dimeric/monomeric forms.[17] The oligomeric state of PKM2 is tightly regulated by different posttranslational modifications (PTMs), including phosphorylation,[18] acetylation,[19] SUMOylation,[20] O-GlcNAcylation,[21] methylation,[22] oxidation,[23] and sulfhydration.[24] These modifications of PKM2 slow down its enzymatic activity, thus redirecting the use of glucose from energy production to biomass synthesis, thereby allowing tumor cells to proliferate faster.

In addition to its role in glycolysis, the dimer and monomer forms of PKM2 are predominant in cancer cells. Dimeric/monomeric PKM2 plays a crucial role as a protein kinase and transcriptional coactivator in the nucleus to promote cancer cell proliferation by modulating cancer metabolism and regulating gene expressions.[25,26] Through interaction with hypoxia-inducible factor-1α, nuclear PKM2 transactivates the expressions of genes such as lactate dehydrogenase A (LDHA), phosphoinositide-dependent protein kinase 1, hexokinase 1, and vascular endothelial growth factor A, promoting cell proliferation and angiogenesis.[26,27] PKM2 forms a complex with β-catenin, binding to the cyclin D1 (CCND1) promoter region to phosphorylate histone H3, which upregulates CCND1 expression.[28] PKM2 also interacts with octamer-binding transcription factor 4, a nuclear protein involved in cancer cell self-renewal and differentiation, thereby enhancing its transcriptional activity.[29]

In conclusion, PKM2 functions not only as a PK enzyme in its tetrameric form but also as a transcriptional coactivator when it transitions to its dimeric or monomeric form and enters the nucleus to mediate gene transcription. The oligomeric state of PKM2 plays a critical and indispensable role in regulating the Warburg effect on cancer cells.

Hydrogen Sulfide

Hydrogen sulfide (H_2S), a colorless, flammable, and water-soluble gas, is the third and most recently identified gasotransmitter.[30] Similar to nitric oxide and carbon monoxide, H_2S serves as a critical mediator in various physiological processes, including the regulation of blood vessel vasodilation, cardiac responses to ischemia, and inflammation.[31-35] In mammalian cells, H_2S is endogenously produced by three different enzymes: cystathionine β-synthase (CBS), cystathionine γ-lyase (CTH), and 3-mercaptopyruvate sulfurtransferase.[30,36] Accumulating evidence has shown the upregulation of H_2S-producing enzymes in multiple cancer types,[37] suggesting that H_2S may play a role in cancer development.

Hydrogen sulfide modulates various cellular signaling pathways mainly through protein sulfhydration, which occurs at reactive cysteine residues of target proteins.[38] This reversible PTM covalently adds a thiol group (-SH) to active cysteine residues (PSH/PS^-) in target proteins, resulting in protein sulfhydration ($PSSH/PSS^-$).[39] A direct reaction on cysteine residues with H_2S is thermodynamically unfeasible. Potential pathways for sulfhydration modifications include the reaction of S-sulfenylated (PSOH),[40] S-Nitrosated (PSNO),[41] or protein disulfides (PSSP)[42] with H_2S, resulting in the formation of sulfhydrated proteins. Following sulfhydration, changes in the spatial arrangement of the modified protein structure play a crucial role in modulating protein functions, such as facilitating the association/dissociation of oligomeric states or promoting protein–protein interactions.[32,43-55] On the other hand, sulfhydration can protect critical cysteine residues from overoxidation during oxidative stress, preventing irreversible damage caused by unfolded protein accumulation.[56] The potential mechanism for protein sulfhydration is summarized in Figure 2.

In cancers, H_2S contributes to progression in multiple aspects. It has been shown to participate in antiapoptotic pathways in multiple cancer types[57-60] by scavenging reactive oxygen and nitrogen species, leading to profound antioxidant protection in cells to promote cancer cell survival.[61] H_2S also aids in DNA repair by activating PARP1, protecting cells from senescence.[47] Cancer cells promote tumor growth through H_2S-mediated sulfhydration on MEK1.[47] In addition, H_2S contributes to cancer metastasis and angiogenesis by facilitating cell migration and invasion, primarily through nuclear factor kappa B modulation.[62] Overall, these findings highlight that H_2S is a versatile mediator in cancer progression, impacting various hallmarks of cancer, including antiapoptosis, DNA repair, tumor growth, metastasis, and angiogenesis.

Hydrogen Sulfide in Cancer Metabolism

Exogenous H_2S has long been recognized as an environmental toxin due to its ability to inhibit mitochondrial complex IV, thereby suppressing mitochondrial electron transport and hindering aerobic ATP production.[63] In contrast, endogenously produced H_2S serves as a metabolic substrate in mitochondria

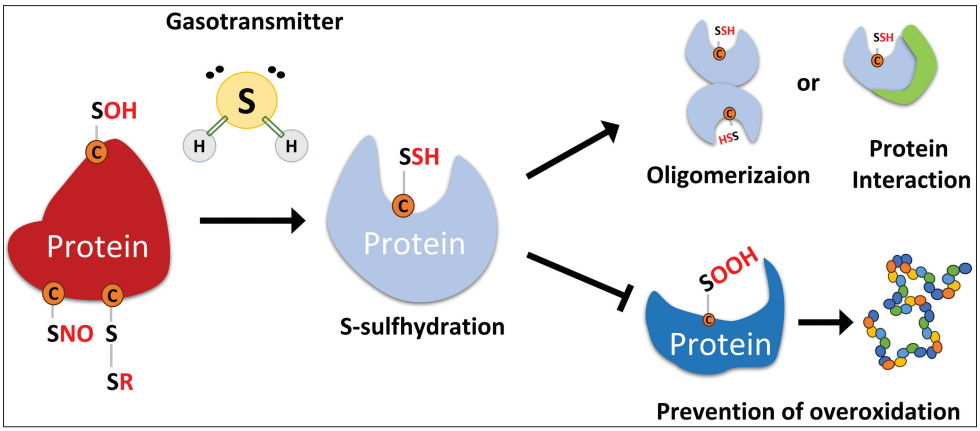

Figure 2: Potential mechanisms for protein sulfhydration. Proposed formation processes for sulfhydration by H_2S as the sulfide donor. H_2S can react with sulfenylated (PSOH), nitrosated (PSNO), or disulfide bonds (PSSR) on cysteine residues to form sulfhydrated proteins (PSSH). Sulfhydration alters protein structure to modulate functions such as oligomeric state transitions and protein–protein interactions, while also protecting critical cysteine residues from irreversible overoxidation damage due to unfolded protein accumulation

to stimulate the mitochondrial electron transport chain and influence cellular metabolism.[64] H_2S is oxidized by sulfide–quinone oxidoreductase (SQR), a component of mitochondrial respiratory complex II anchored in the inner mitochondrial membrane. This process transfers electrons to coenzyme Q (CoQ), thereby facilitating aerobic ATP synthesis through the respiratory chain.[64] In addition, H_2S also increases the catalytic activity of mitochondria ATP synthase through sulfhydration on the α subunit of ATP synthase (ATP5A1),[65] which leads to increased ATP production in mitochondria through enhanced aerobic respiration. The role of H_2S-stimulated mitochondrial respiration in cancer progression remains unclear, as cancer cells typically favor glycolysis even when sufficient oxygen is available.

On the other hand, depletion of H_2S production by CTH knockout or H_2S synthesis inhibitors has been shown to lead to an increase in oxygen consumption in endothelial cells[66] and pancreatic beta cells.[67] In these studies, treatment with H_2S reduced OXPHOS while enhancing lactate production, glucose uptake, and glycolytic ATP production.[66,67] This metabolic shift from OXPHOS to glycolysis indicates that H_2S may play a role in regulating the Warburg effect. Another study further suggested that L-cysteine, an endogenous substrate for H_2S production,[68] could destabilize tetrameric PKM2 into the dimeric/monomeric form.[69] A recent study by Wang *et al*.[24] further demonstrated that H_2S modulates PKM2 activity through protein sulfhydration, causing dissociation of the highly active tetrameric form into the less active dimeric form. Conversely, blocking PKM2 sulfhydration at cysteine 326 through mutation prevented tetramer dissociation, thereby promoting OXPHOS for high-energy production and reduced biosynthesis, ultimately leading to significant tumor growth inhibition [Figure 3]. In addition to modulating PKM2 activity, H_2S-mediated sulfhydration has been shown to enhance the enzyme activity of LDHA[70] and glyceraldehyde 3-phosphate dehydrogenase (GAPDH).[39,67] Taken together, sulfhydration of PKM2, LDHA, and GAPDH by H_2S induces changes in protein dynamics on these glycolytic enzymes to induce the Warburg effect and promote tumor growth.

Conclusion and Perspectives

Metabolic reprogramming is a hallmark of cancer,[71] in which the Warburg effect is essential to support the proliferation and malignant progression of certain cancer types.[72] As cancer cells are dependent on metabolic reprogramming to survive and proliferate, targeting these altered pathways is an attractive approach for anticancer drug development. However, since normal proliferating cells, such as immune cells, share similar metabolic rewiring mechanisms with cancer cells, identifying a therapeutic approach that can distinguish between the two may be a significant challenge in developing effective cancer therapies targeting metabolic pathways.

On the other hand, dysregulated upregulation of H_2S-producing enzymes has been frequently observed in various cancer types, including breast, prostate, colon, ovarian, gastric, bladder, thyroid, and gallbladder cancers, as well as glioma,[37] where it contributes to cancer progression.[73] Targeting H_2S production in these cancers offers a promising avenue for drug development. However, current options for inhibiting endogenous H_2S production remain highly limited. The most commonly used inhibitor, DL-Propargylglycine (PAG), is an irreversible inhibitor of CTH with high selectivity for CTH over CBS.[74] Unfortunately, PAG is not clinically viable due to its poor cell permeability.[75] Another commonly used inhibitor, aminooxyacetic acid (AOAA), inhibits both CBS and CTH;[76] however, its specificity is low as it broadly inhibits several other PLP-dependent enzymes.[77] Despite the therapeutic potential of targeting H_2S production in cancer, concerns about off-target effects and systemic toxicity present significant challenges. In particular, broad-spectrum inhibitors such as AOAA may lead to unintended disruption of critical metabolic pathways to impact cellular functions, increasing the risk of toxicity.[78] Therefore, the development

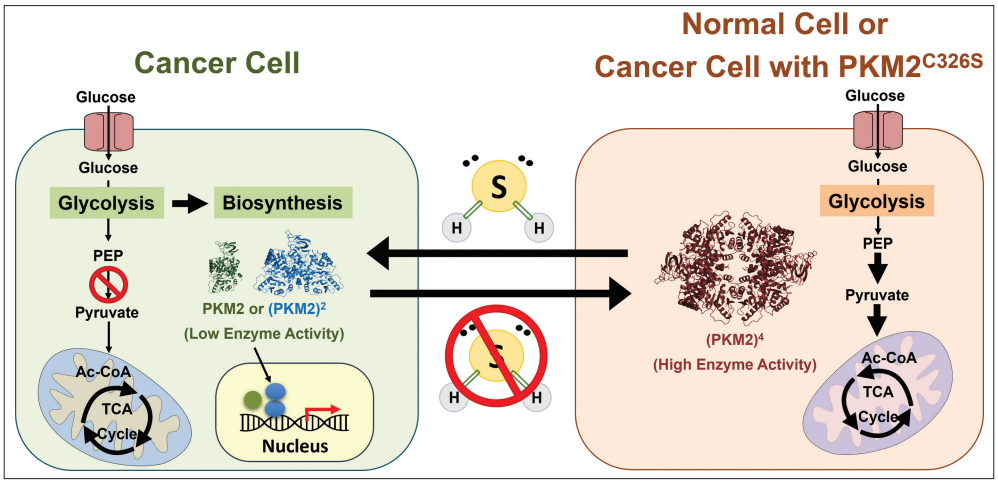

Figure 3: H_2S modulates glucose metabolism switch through destabilizing tetrameric pyruvate kinase M2 (PKM2) into dimer or monomer. In cancer cells, H_2S promotes the dissociation of the high enzyme activity PKM2 tetramer into low enzyme activity monomers/dimers through protein sulfhydration. Inhibition of PKM2 activity leads to the accumulation of glycolytic intermediates required for biosynthesis. Meanwhile, the PKM2 monomers/dimers translocate to the nucleus to activate gene expression, promoting rapid cancer cell proliferation and tumor growth. In normal differentiated cells or cells with PKM2 sulfhydration blocked at cysteine 326, PKM2 remains stabilized as a tetramer with high PK activity, resulting in high energy production, low biosynthesis, and inhibition of cell proliferation

of highly specific inhibitors is needed and must be approached with caution, balancing therapeutic efficacy against potential adverse effects arising from systemic H_2S depletion and nonspecificity.

Considering the critical role of H_2S-mediated sulfhydration in regulating the activities of PKM2 and other glycolytic enzymes, targeting specific sulfhydrated cysteine sites, such as cysteine 326 on PKM2, could provide a cancer-specific therapeutic strategy that minimizes toxicity to normal tissues. Developing small molecules that block PKM2 sulfhydration may effectively shift cancer cell metabolism from glycolysis back to OXPHOS, ultimately suppressing tumor growth.

Looking ahead, a deeper understanding of the biochemical pathways driving cancer metabolism will help identify the most promising therapeutic targets. This knowledge will pave the way for the development of highly selective and potent pharmacological inhibitors targeting the Warburg effect, offering new treatment options to benefit a broader range of cancer patients.

Data availability statement

This study did not involve the generation or analysis of data.

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Conflicts of interest

There are no conflicts of interest.

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