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Non Canonical Roles of the Metabolic Enzymes PKM2, ENO1 and GAPDH as Drivers that Facilitate the Adaptation of Glioblastoma Cells to Stress, Promoting Malignant Progression.

Deepti Prabhakar
deeptiprabhakar789@gmail.com

ABSTRACT

Glioblastoma Multiforme (GBM) is an aggressive, treatment-resistant brain tumor that uses metabolic plasticity to support its survival and progression. This paper explores how the glycolytic enzymes PKM2, ENO1 and GAPDH take on non canonical functions outside of its primary metabolic role in glycolysis in GBM cells. PKM2 enters the nucleus to activate transcription factors like HIF-1 alpha which drive the expression of genes involved in proliferation, angiogenesis and glucose metabolism. ENO1 promotes the activation of the P13K/ Akt pathway and supports immune evasion by suppressing MHC class 1 expression and supporting polarisation of M2-like macrophages. GAPDH, another glycolytic enzyme, also responds to stress by moving into the nucleus where it regulates the activity of a protein p53 and sometimes is involved in acetylation though interactions with p300 CBP which in turn influences both cell cycle arrest and apoptosis. Together these enzymes help the tumour adapt to stress in the harsh tumour microenvironment and maintain its aggressive behaviour. Their functions show how metabolism and gene regulation are interconnected in GBM and suggest that targeting these moonlighting roles may offer new therapeutic strategies that show promise.

INTRODUCTION

In oxygen deficient environments, the anaerobic pathway of respiration is chosen due to fast production of ATP molecules even though the number of molecules of ATP synthesised per cycle is much lower (Melkonian and Schurry, 2023). In cancer cells however, even in an environment with an abundant supply of oxygen, oxidative phosphorylation (the aerobic pathway) is not chosen. Only glycolysis is repeatedly carried out for the fast supply of energy and so that the biosynthetic intermediates formed in the process can be reprogrammed to be used for cell proliferation and development of the cancer (Vaupel and Mayer, 2019). This metabolic reprogramming is called the Warburg effect and it is the process whereby cancer cells favor glycolysis even in an oxygen rich environment resulting in an overexpression of glycolytic enzymes (Vaupel et al., 2019). Apart from their canonical role in glycolysis that occurs in the cytoplasm,

they start to develop non canonical functions. Both "non-canonical" and "moonlighting" functions refer to enzymatic activities outside their primary metabolic role, referring to proteins performing multiple independent tasks, often unrelated to their original biochemical function (Singh & Bhalla, 2020). These functions contribute to the development of long term adaptations in tumour cells allowing them to resist therapy and survive (Alberghina, 2023). However it is important to note that relocation of these enzymes from the cytoplasm to the different parts of the cell occurs only when the cell is under stress (Alberghina, 2023). This relocation is due to a broader principle of stress adaptation, where metabolic reprogramming allows cells to survive nutrient deprivation, hypoxia, or DNA damage. Understanding this framework helps situate glycolytic enzyme multifunctionality as a key adaptive strategy in cancer biology.

Cellular stressors refer to the individual characteristics of harsh environments in which tumour cells grow including but not limited to: nutrient shortage, DNA damage and hypoxia (Erasimus et al., 2016; Vijayanathan & Ho, 2025; Colwell et al., 2016). Such stressors force cancer cells to adapt and hence give rise to the relocation of glycolytic enzymes into the nucleus and other regions consequently enabling them to perform their non canonical functions (Rodríguez-Saavedra et al., 2021). Different types of cancer experience a different combination of stressors. One of the types of cancer that has a very harsh tumour microenvironment is Glioblastoma. It is consequently especially good at adapting and surviving under stress(Bailleul & Vlashi, 2023).

Glioblastomas (GBMs) are a common and the most malignant type of brain cancer. The average annual incidence rate of GBMs are 3.19 per 100,000 persons in the US and between 0.59 and 3.68 per 100,000 persons in other countries. The median survival rate even with clinical intervention is around 15% and even then, less than 5% of all patients survive more than 5 years after diagnosis. They make up 54% of gliomas diagnosed and up to 16% of all brain tumours (Tamimi & Juweid, 2017). Gliomas are primary brain tumours that arise from glial cells in the brain. Glial cells include: astrocytes, oligodendrocytes and ependymal cells (Weller et al., 2015) . Glioblastomas are grade IV astrocytomas (Hanif et al., 2017). They have a very harsh tumor microenvironment and their tumour cells experience many of the cellular stressors mentioned above (Bailleul & Vlashi, 2023). There are several reasons for this. Firstly, GBMs grow very fast and consequent angiogenesis (formation of new blood vessels) is unable to keep up at the same speed. This leads to a shortage in blood supply and hence creates a hypoxic environment for tumour cells (Kaur et al., 2005). Due to this, Glioblastomas becomes more adaptive: they switch to anaerobic metabolism (glycolysis) for a faster supply of energy. Secondly, because the brain is in the skull (a closed space), as the tumour grows, intracranial pressure builds. This pressure compresses blood vessels in the brain that are once again providing both oxygen and nutrients to tumour cells. Because these vessels are now compressed, it leads to the expression of two cellular stressors: Nutrient shortage and hypoxia once again (Cavazos & Brenner, 2015; Arvanitis et al., 2019). The exposure of GBM's to radiation therapy also causes DNA damage to tumour cells. Instead of going into programmed cell death (apoptosis),

GBMs adapt by the non canonical function of glycolytic enzymes such as PKM2, ENO1 and GAPDH where they support DNA repair and alter gene expression to promote survival even in the harsh tumour

microenvironment. In this case, they protect the cells from oxidative stress which is caused by radiation (Bailleul et al., 2023). This review focuses on PKM2, ENO1, and GAPDH because they are the glycolytic enzymes most consistently found in the nucleus and other regions of glioma cells and together they help GBM cells to survive and adapt to stress. Their non-canonical functions affect gene expression, chromatin regulation, and DNA repair (Yang et al., 2011; Zheng et al., 2019; Mikeladze et al., 2021). These multifunctional behaviors are said to have evolved to optimize cellular energy efficiency by diverting metabolic intermediates into biosynthetic pathways, maintain redox balance through modulation of reactive oxygen species, and increase adaptive plasticity by enabling dynamic responses to hypoxia, nutrient deprivation, and DNA damage (Singh & Bhalla, 2020). While other enzymes may also translocate under certain conditions, these three are more commonly active and are supported by experimental studies. Despite extensive studies on individual glycolytic enzymes, there remains a lack of integrated understanding of how multiple non-canonical enzyme functions collectively contribute to GBM stress adaptation; this review aims to address this gap by synthesizing the integrated roles of PKM2, ENO1, and GAPDH.

METHODOLOGY

For this literature review, sources were identified through searches on PubMed and Google Scholar using keywords such as “PKM2 moonlighting in GBM,” “Metabolic reprogramming and Glioblastoma,” and “Non canonical roles of metabolic enzymes in glioblastoma.” Only studies published in the last decade were considered, with few older foundational papers included for context. Selection criteria focused on relevance to non-canonical enzyme functions and their role in glioblastoma metabolism. Additionally, reference lists of the identified papers were examined to uncover further relevant studies, expanding the scope and ensuring comprehensive coverage of the topic. This approach allowed for a focused yet comprehensive overview, situating the review within the broader context of metabolic reprogramming and multifunctional enzyme behavior in glioblastoma.

REVIEW BODY

4.1: Glioblastoma Multiforme (GBM)

Glioblastomas are tumours that form in the astrocytes. It is most commonly located on the supratentorial region of the brain with the highest incidence being on the frontal lobe followed by overlapping lobes, parietal lobe and temporal lobe (Tamimi & Juweid, 2017). It is more predominant in males with the male to female ratio being 1:0.33. Age adjusted GBM incidence rate is also higher in European Americans by 2.5 times more than African Americans. While the majority of GBM cases are sporadic, several heritable syndromes like Turcot’s syndrome, Li Fraumeni syndrome, neurofibromatosis and tuberous sclerosis complex significantly increase lifetime risk. Better prognosis for GBM’s only occurs from complete tumor resection. Worse prognosis is associated with age and tumors in eloquent areas like the motor cortex (Tamimi & Juweid, 2017).

Among all environmental risk factors, ionizing radiation is the only one that has been consistently validated. Specifically, this refers to individuals who underwent therapeutic cranial irradiation during childhood (Abuhamed et al., 2024). In contrast, lifestyle factors such as smoking, alcohol, mobile phone usage and occupational electromagnetic fields do not exhibit consistent associations with GBM (Gheorghiu et al., 2024). Interestingly, there have been studies that show that atopic and allergic conditions like asthma, eczema, hay fever etc are statistically notable to have an inverse correlation with glioma risk. Controlled studies indicate that individuals with an allergic history show a 40% reduction in glioma incidence. Following this, genome wide association studies (GWAS) have shown that single nucleotide polymorphisms near genes involved in IgE-mediated hypersensitivity are inversely associated with glioma risk (Dobbins et al., 2010).

On a genomic level, glioblastomas are characterized by a complex environment of somatic mutations, copy number alterations and epigenetic changes that drive tumour initiation and subsequent progression. According to data from the TCGA (The Cancer Genome Atlas), core signalling pathways disrupted in GBM include: P13K/Akt, TP53 and Rb pathways . The most commonly altered genes are TP53 and EGFR (Cerami et al., 2010; Ding et al., 2022).

GBM is treated using a multimodal strategy combining maximal safe surgical resection along with chemotherapy and radiation. This regimen, known as the Stupp protocol, remains as the gold standard (Obrador et al., 2024). Surgical resection offers cytoreductive benefits and symptom relief. Extent of resection is directly correlated with prognosis (X. Li et al., 2017). This followed by adjuvant radiotherapy and cycles of chemotherapy. Despite extensive treatment, most tumours recur due to intratumoural heterogeneity, therapy resistance and immunosuppressive environments (Goenka et al., 2021).
In response to this therapy resistance, new forms of emerging therapies are being investigated. For example, tumour treating fields (TTFs) are a non-invasive device that delivers alternating electrical fields to disrupt the formation of mitotic spindles in the cell cycle. This has gained FDA approval and has been seen to improve survival at small rates when combined with other therapies (Regev et al., 2021). Looking at it at a molecular level, many targeted inhibitors against EGFRvIII, IDH1/H2, VEGF and PDGFR are in various stages of trials. Such inhibitors look to stop angiogenesis, cell proliferation and adaptive behaviours of GBM's to stress (Tamimi & Juweid, 2017). For example, Bevacizumab, a VEGF inhibitor, can alleviate peritumoral edema but has not consistently altered survival (Omar, 2014). Still, the median survival period remains to be 15 months after diagnosis (Tamimi & Juweid, 2017). Further research into targeting non canonical functions of certain enzymes like PKM2, GAPDH and ENO1 that influence metabolic plasticity and therapy resistance are future directions to better treat Glioblastomas .

4.2: PKM2 (Pyruvate Kinase M2)

In the glycolysis pathway shown in Fig 1, it can be clearly observed that the enzyme pyruvate kinase M2 catalyses the last step in the pathway converting PEP (Phosphoenolpyruvate) to pyruvate (Melkonian and Schury, 2023). This essential step also results in the phosphorylation of one ADP molecule to ATP (Melkonian & Schury, 2023). It is hence one of the most important steps in the pathway and is one that is

heavily relied on in cells performing glycolysis. Pyruvate kinase has many isoforms. Pyruvate Kinase M2 is specifically expressed in Glioblastoma cells over PKM1 which is the default isoform in most adult brain cells. Transcriptomic and proteomic analyses confirm that PKM2 is markedly overexpressed in glioblastomas, showing approximately a three to five-fold increase in RNA and protein levels compared to low-grade gliomas and normal brain tissue (Mukherjee et al., 2013). Elevated PKM2 expression also correlates with poorer overall survival in GBM patients, suggesting its upregulation contributes to tumor aggressiveness (Stanke et al., 2021). PKM1 is a continuously active isoform that pushes glucose carbons into the glycolysis pathway for ATP production (Alberghina, 2023). PKM2 on the other hand can switch between two conformations: one that is active (its tetrameric conformation) and one that is much more passive (its dimeric conformation). This dual conformational ability distinguishes PKM2 from PKM1, which remains constitutively active. While PKM1 ensures steady ATP output, PKM2's capacity to alternate between tetrameric and dimeric states allows glioblastoma cells to fine-tune their metabolism according to stress conditions. This adaptive flexibility contrasts the rigid energy-producing role of PKM1 and is central to the metabolic plasticity observed in GBM. In its tetrameric conformation, PKM2 acts much like PKM1 where it promotes rapid glycolysis. On the other hand, its dimeric isoform is a less active enzyme that slows glycolysis and pushes intermediates of glycolysis into biosynthetic pathways (Bailleul & Vlashi, 2023). This low activity dimeric form is expressed in glioblastomas which supports the Warburg effect to prioritise biomass production for growth over efficient energy production (Bailleul & Vlashi, 2023). By expressing PKM2 over PKM1, Glioblastoma cells develop a metabolic plasticity where they adapt for better survival (Bailleul et al., 2023).

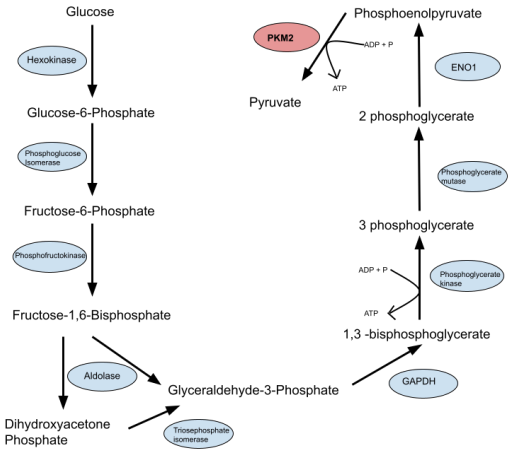

Figure 1: PKM2 in the glycolytic pathway

However, PKM2 does not just influence metabolism of these cells, they have many non canonical functions that they carry out in the nucleus. One mechanism that influences this is the EGFR (Epidermal Growth Factor Receptor) mutation. Such mutations are general hallmarks of many cancers including glioblastomas (Yang et al., 2011; Luo & Semenza, 2012). In normal cells, EGFR is only activated when external growth factors are also present, signalling a necessary growth in the human body. However the

mutated form of EGFR in glioblastomas continuously signals for growth even without the availability of external growth factors (Luo & Semenza, 2012) .

This does not occur on its own but instead it is part of a bigger adaptation to cellular stress. GBM's grow rapidly and outgrow their blood supply creating many regions of hypoxia, nutrient deprivation, reactive oxygen species and acidic pH within the cell. Parallely, rapid repetitions of the cell cycle also cause DNA damage. All these conditions would typically pause the cell cycle and cause apoptosis.

In GBMs however, they adapt by expressing more of the low activity dimeric form of PKM2 instead of the tetrameric one. This lacks efficient glycolytic function but has many regulatory functions. In low oxygen conditions, enzymes like prolyl hydroxylase are inhibited causing the (Yang et al., 2011). This not only drives glycolytic gene expression but also causes the nuclear translocation of PKM2. In the cytoplasm, the HIF-1alpha factor binds to PKM2 carrying it to the nucleus where together they bind to promoter regions on genes and act as a coactivator for genes that induce angiogenesis, reduce mitochondrial oxygen intake, increase glycolysis etc all of which are genes that promote tumour survival in stress (Yang et al., 2011).

Additionally, under EGFR cosignaling, PKM2 also interacts with oncogenic transcription factors like beta catenin. The mutated, activated EGFR in glioblastomas activates a phosphorylation cascade with kinases like c-Src (often upregulated in cancer cells) (Luo & Semenza, 2012). This directly phosphorylates the protein beta catenin. This specific phosphorylation at the location of tyrosine residue 333 causes conformational changes to beta catenin allowing it to translocate to the nucleus and bind to DNA promoters and transcriptional coactivators in the nucleus (Luo & Semenza, 2012).

Under the influence of both cellular stress and EGFR signalling, PKM2 dimer has already accumulated in the nucleus. Here, it binds with the newly translocated phosphorylated beta catenin and forms a complex that binds to the promoters of two major oncogenes: CCND1 which codes for the cyclin D1 that pushes the cell cycle forward from the G1 checkpoint (where in normal cells, the cycle stops if DNA damage is detected). Secondly, it binds to the promoter of the MYC gene that codes for c-Myc which is a regulator of cell growth and proliferation (Luo & Semenza, 2012). PKM2 modifies chromatin and enhances the transcription activity of beta catenin at these promoter sites. With both these oncogenes influenced by this complex, tumour cell proliferation is directly amplified, adapting to the cellular stressors like hypoxia in the GBM microenvironment (Luo & Semenza, 2012). Bailleul & Vlashi (2023) discuss how PKM2's dimeric form supports metabolic reprogramming in cancer cells, while Luo & Semenza (2012) focus on its role as a transcriptional coactivator under stress conditions. Yang et al. (2011) complement this by showing that under hypoxia, PKM2 nuclear translocation alongside HIF-1α enhances transcription of genes that promote angiogenesis and glycolysis. All studies agree that dimeric PKM2 supports metabolic plasticity, but they differ in the primary mechanism highlighted.

Similar to PKM2, other glycolytic enzymes like ENO1 also exhibit multifunctional behavior beyond metabolism.

4.3: ENO1 (Alpha enolase)

ENO1 (alpha enolase) is another glycolytic enzyme that catalyses the second last step of glycolysis: the conversion of 2 phosphoglycerates to phosphoenolpyruvate (PEP) as shown in fig 2. It acts one step before PKM2 and is also vital to the process of glycolysis (Melkonian & Schury, 2023). ENO1, like PKM2, also performs many non canonical functions in Glioblastoma cells (Li et al., 2024). In fact, Li et al. (2024) report that ENO1 levels in GBM are roughly 2-3 times higher than in normal glial cells, highlighting its strong upregulation in tumour cells. As mentioned previously, the GBM tumor microenvironment experiences many cellular stressors. Under such conditions, ENO1 translocates to the nucleus (Zheng et al., 2019). While Zheng et al. (2019) identify hypoxia as the primary trigger for nuclear localisation, Trejo-Solis et al. (2023) extend this by showing that nuclear accumulation also results from metabolic stress, suggesting ENO1 responds to multiple microenvironmental cues.

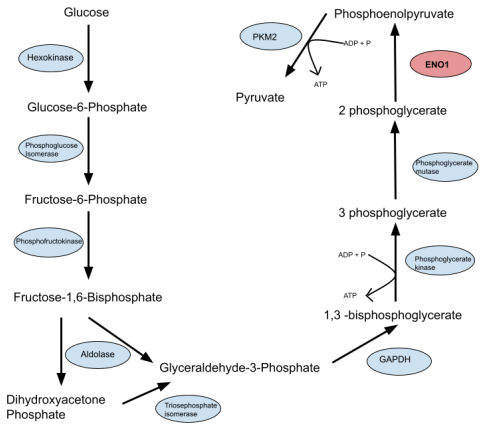

Figure 2: ENO1 in the glycolytic pathway

Once inside the nucleus, ENO1 activates the PI3K/ Akt pathway which is a signalling pathway that promotes cell proliferation. This also upregulates Cyclin D1 and E which push the cell cycle past the checkpoint that controls and stops cell division (Trejo-Solis et al., 2023). A domain protein called WW Domain binding protein 2 (WBP2) binds directly to ENO1 strengthening the activation of the PI3K/ Akt pathway (Chen et al., 2018). These two mechanisms work in synergy (Trejo-Solis et al., 2023). Chen et al. (2018) focus on ENO1-WBP2 binding as a structural driver of pathway amplification, whereas Trejo-Solis et al. (2023) interpret this interaction functionally, linking it to proliferation and EMT, indicating complementary mechanistic insights. Subsequently it activates a transcription factor called NF-KB that promotes tumour inflammation and survival. ENO1 also contributes to the inactivation of Rb by hyperphosphorylation. When it is active, Rb (Retinoblastoma) also stops the cell cycle from uncontrolled division. Its inactivation allows uncontrolled proliferation of GBM cells. This environment where NF-KB is activated and Rb is inactivated, promotes a hallmark of most cancers including GBM: EMT(Epithelial to Mesenchymal transition). This is a process by which cells develop motility. ENO1

facilitates this process more through increased gene expression that codes for Slug, Snail, Vimentin and N-Cadherin all of which contributes to the general GBM proliferation and EMT (Trejo-Solis et al., 2023). Yang et al. (2022) further report that ENO1 expression rises under hypoxic conditions, reinforcing its coupling with HIF-1α signalling and predicting reduced median survival (13.6 vs 20.2 months), confirming its prognostic significance.

Additionally, overexpression of ENO1 is linked to increased expression of ACL (ATP citrate lyase) which promotes lipid biosynthesis promoting the GBM tumour's metabolism (Trejo-Solis et al., 2023).

In the nucleus, ENO1 also influences the evasion of the body's immune response to tumour cells.
ENO1 expression is associated with reduced MHC (Major Histocompatibility Complex) class 1 expression which impairs the tumour antigen presentation to cytotoxic CD8+ t cells (Tulamaiti et al., 2025). T cells are immune cells in our body that target antigens of foreign cells in the body (like viruses, cancer cells etc) and then they kill these cells. So when ENO1 creates an immunosuppressive environment by impairing the antigen presentation of tumours to T cells, the immune response of the body is evaded (Tulamaiti et al., 2025). ENO1 also promotes M2-like polarisation macrophages which boosts angiogenesis, immune tolerance and tissue remodeling (Liang et al., 2022). This is done instead of M1-like macrophage polarisation which would have promoted anti tumour immune response (Ren et al., 2023). Quantitatively, ENO1 expression shows a strong positive correlation with macrophage infiltration ($r = 0.58$, $p < 0.001$) in TCGA GBM cohorts (Liang et al., 2022). While Tulamaiti et al. (2025) highlight this link mainly in the context of adaptive immune evasion, Liang et al. (2022) and Ren et al. (2023) broaden this understanding by showing that ENO1 also reprograms innate immunity through macrophage polarization. Together, they show that ENO1 suppresses both adaptive and innate responses, creating a layered immunosuppressive environment that favours tumour progression.

A key difference to note at this point is that the non-canonical functions of ENO1 are not restricted solely to the nucleus like PKM2. Long non-coding RNA SNHG18 regulates the localization of ENO1 (Trejo-Solis et al., 2023). In GBM cells, when overexpression of this RNA strand occurs, nuclear translocation is inhibited and ENO1 accumulates once again in the cytoplasm. However, it does not just assume the role of a glycolytic enzyme again(Trejo-Solis et al., 2023). Accumulation in the cytoplasm is associated with increased motility and invasiveness of all glioma cells including GBMs. Interestingly, Zheng et al. (2019) report that when ENO1 is retained in the nucleus, glioma cells show reduced migration. This contrast implies that ENO1's localisation determines its function promoting proliferation when nuclear, but invasion when cytoplasmic. Experiments that silence cytoplasmic ENO1 reduce these effects, confirming that cytoplasmic ENO1 promotes invasion. This leaves the possibility that nuclear ENO1 despite all that it does to promote GBM survival actually prevents invasion (Trejo-Solis et al., 2023).

ENO1 also has extracellular moonlighting roles. Some ENO1 is translocated to the cell membrane of GBM cells. Beyond its intracellular functions, ENO1 also operates extracellularly. Kumari and Malla (2015) demonstrate that its role as a plasminogen receptor connects metabolic enzymes to tissue remodelling and invasion, aligning with Trejo-Solis et al. (2023)'s observation of its contribution to GBM aggressiveness. Here, at the cell surface, they act as plasminogen receptors. Plasminogen circulates in the extracellular space and is the inactive form of plasmin (a protease that breaks down structural proteins in cell membranes) (Trejo-Solis et al., 2023). Once binding takes place, plasminogen is then converted to plasmin by specialised activator enzymes called tPA (tissue type plasminogen activator) and uPA (Urokinase-type plasminogen activator) (Kumari & Malla, 2015). These are proteases in the extracellular environment that activate plasminogen to plasmin. These proteases are often upregulated in cancer cells which is vital as ENO1 cannot make the conversion by itself (Kumari & Malla, 2015). Once activated, plasmin begins digesting components of the extracellular matrix (ECM) which is the complex network of molecules that hold cells in place. This removes physical barriers so that these GBM cells can invade neighboring brain tissue and migrate easily (Trejo-Solis et al., 2023). This is a characteristic of GBM that makes it very aggressive.

Alongside PKM2 and ENO1, GAPDH likewise demonstrates extensive non-canonical activity, highlighting a broader pattern of metabolic reprogramming in GBM.

4.4: GAPDH (Glyceraldehyde-3-Phosphate Dehydrogenase)

As seen in the figure shown below, GAPDH (Glyceraldehyde-3-Phosphate dehydrogenase) catalyses one of the the crucial steps part of ATP synthesis by glycolysis: the conversion of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate (Melkonian & Schury, 2023). In glioblastomas however this glycolytic enzyme takes on numerous non canonical roles similar to both PKM2 and ENO1.

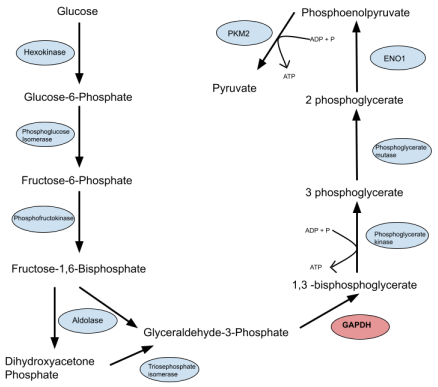

Figure 3: GAPDH in the glycolytic pathway

In GBMs, GAPDH is significantly overexpressed as compared to its levels in low grade gliomas and non cancerous brain tissue. Quantitatively, proteomic data show roughly a 2-2.5 fold increase in GAPDH

expression relative to normal tissue (Trejo-Solis et al., 2023), reinforcing that metabolic upregulation is not incidental but functionally selected in high-grade gliomas. This suggests that the function of GAPDH boosts cell proliferation and is essential to attain the malignancy posed by GBMs (Trejo-Solis et al., 2023). Specifically, TAMs (Tumour Associated Macrophages) secrete interleukin 1beta (IL-1beta) which activates intracellular signalling pathways that phosphorylate GAPDH thereby activating it. This increases glycolytic efficiency and thereby reinforces the Warburg effect where intermediates are redirected to fuel cell proliferation further (Trejo-Solis et al., 2023). While Trejo-Solis et al. (2023) describe cytokine-driven phosphorylation that enhances GAPDH activity, Mikeladze et al. (2021) highlight its stabilisation through Hsp-70 binding under hypoxia. Together, these findings suggest that GAPDH persists in GBMs through both metabolic activation and structural protection, allowing it to sustain glycolysis even under stress.

Under hypoxia, GAPDH expression increases and it forms a complex with the cellular molecule Hsp-70 in glioblastoma cells (Mikeladze et al., 2021). This interaction stabilises GAPDH and prevents its aberrant aggregation under stress (which would otherwise disrupt its glycolytic function that is essential in cancer cells as they promote the Warburg effect) (Mikeladze et al., 2021). This complex formed between GAPDH and Hsp-70 helps maintain proteostasis which is the cell's ability to maintain proper balance, folding, function and turnover of proteins (Mikeladze et al., 2021). This adaptation to stress allows GAPDH to serve its metabolic function in glioblastoma cells helping it sustain glycolysis and allowing subsequent metabolites to be used in the tumour cell . Importantly, Hsp-70 also inhibits both caspase dependent apoptotic pathways and caspase independent ones by neutralising pro-apoptotic pathways like Bax, TRAIL (Tumour Related Apoptosis Inducing Ligand) and AIF (Apoptosis inducing factor) (Trejo-Solis et al., 2023).

Interestingly, not all stress responses involving GAPDH promote survival. Zhang et al. (2015) identify its nuclear acetylation as a switch toward pro-apoptotic signalling, which contrasts with Trejo-Solis et al. (2023)'s survival-oriented view of cytoplasmic activation. This contrast underscores GAPDH's dual regulation under varying stress intensities. Once nuclear and still under stress, GAPDH can also be acetylated at Lysine 160 by the histone acetyl transferase p300/CBP (Zhang et al., 2015). This post translational modification of the histone acetyl transferase enhances its enzymatic activity of the transcriptional coactivator p300/CBP through a positive feedback loop. The result of this is increased transcription of p53. Specifically, activation of p300 and p53 results in the upregulation of certain genes like PUMA and Bax (Trejo-Solis et al., 2023). These are the main activators of the mitochondrial apoptotic pathway which triggers mitochondrial outer membrane permeabilisation, enabling cytochrome release and activating caspase cascades resulting in controlled death of GBM cells (Ge et al., 2018; Tao et al., 2022; Trejo-Solis et al., 2023).

Nuclear GAPDH hence has a dual function as stress sensing transcriptional coactivator and as a trigger for apoptosis. Based on the severity and type of stress, GAPDH leverages one of these functions either promoting survival of the GBM or cell death. GAPDH can localise to the cytoplasm, nucleus, mitochondria and endoplasmic reticulum with its distribution being actively influenced by environmental

stress like hypoxia, reactive nitrogen species (RNS) and DNA damage (Trejo-Solis et al., 2023). Additionally, even its conformation (Tetrameric or Homo-oligomeric) determines its functional outcomes. Post translational modifications like phosphorylation and acetylation also modulate GAPDH activity and compartmentalization (Trejo-Solis et al., 2023). This multiplicity of localisation and modification patterns demonstrates that GAPDH does not function as a static glycolytic enzyme in tumour cells. The convergence across studies including Trejo-Solis et al. (2023), Zhang et al. (2015), and Mikeladze et al. (2021) is that GAPDH adapts according to environmental stress level, determining whether GBM cells undergo adaptation or apoptosis.

4.5: Integrated roles of glycolytic enzymes in GBM survival under stress

	PKM2	ENO1	GAPDH
Canonical Role	It catalyses the last step in the glycolytic pathway converting PEP (Phosphoenolpyruvate) to pyruvate	It catalyses the second last step of glycolysis: the conversion of 2 phosphoglycerate molecules to phosphoenolpyruvate (PEP)	It catalyses the conversion of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate.
Non-canonical Role	PKM2 translocates to the nucleus under hypoxia or EGFR signaling, where it acts as a transcriptional coactivator with HIF-1α and β-catenin.	ENO1 translocates to the nucleus and activates the PI3K/Akt and NF-κB pathways, upregulating Cyclins D1/E and promoting proliferation, inflammation, and EMT while inactivating Rb. It also suppresses immune responses by reducing MHC-I expression and promoting M2 macrophage polarization	GAPDH in glioblastomas is overexpressed and activated by IL-1β signaling, enhancing glycolytic flux. Under hypoxia, it complexes with Hsp-70 to inhibit apoptosis. When translocated to the nucleus, acetylated GAPDH activates p300/CBP and p53, upregulating pro-apoptotic genes like PUMA and Bax.
Adaptation	This non-canonical role supports glioblastoma survival and growth under metabolic stress by promoting angiogenesis and cell proliferation.	Cytoplasmic ENO1 enhances glioma invasiveness, and membrane-bound ENO1 binds plasminogen to generate plasmin, degrading the extracellular matrix and facilitating GBM invasion.	GAPDH acts as both a stress sensor and regulator, toggling between promoting survival or apoptosis depending on cellular stress conditions

Figure 4: Summary of roles of PKM2 ,ENO1 ,GAPDH

All three enzymes are overexpressed in GBMs which is a hallmark that has been linked to poor prognosis and aggressive tumour growth (Trejo-Solís et al., 2024). Their interactions in GBM cells promote biosynthesis, survival pathways, and overall cell proliferation. Each enzyme can translocate to the nucleus under stress (although some of them localise in other cellular areas depending on many factors). In the nucleus, they all act as transcriptional coactivators for different genes (Trejo-Solis et al., 2023). PKM2 binds with HIF-alpha and beta catenin to activate the genes CCND1 and MYC promoting cell cycle progression and proliferation (Yang et al., 2011). ENO1 binds to WBP2 and activates P13K/Akt signalling pathway which upregulates cyclin D1 and E and inactivates Rb for cell cycle progression, and induces EMT (Epithelial to Mesenchymal transition) (Chen et al., 2018; Trejo-Solis et al., 2023). GAPDH, when acetylated by p300 acts as a transcriptional coactivator for pro-apoptotic pathways (Trejo-Solis et al., 2023).

Although all three enzymes act as transcriptional coactivators, in this case, GAPDH, unlike PKM2 and ENO1, activates signals for cell death.

Apart from engaging in synergistic nuclear signalling in stressful environments, they also coordinate suppression of apoptosis and immune evasion. GAPDH, although it can coordinate cell death when acetylated in the nucleus, can also suppress apoptosis through binding with Hsp-70 under stress. This sustains functional glycolysis and inhibits both caspase dependent and caspase independent apoptotic pathways (Trejo-Solis et al., 2023). Similarly, PKM2 and ENO1 enhance signalling pathways like P13K/ Akt and dysregulate expression of Myc (Chen et al., 2018; Luo & Semenza, 2012) . All of this collectively suppresses apoptosis. ENO1 also downregulates MHC-1 (impairing antigen presentation) and pushes TAMs (Tumour Associated Macrophages) to M2-like polarization instead of M1-like polarisation avoiding detection from immune cells and promoting angiogenesis (Tulamaiti et al., 2025; Liang et al., 2022) .

Furthermore, ENO1 which is localised to the GBM cell surface binds and activates plasminogen which facilitates ECM (Extracellular Matrix) degradation promoting tumour invasiveness (Trejo-Solis et al., 2023). All three enzymes, through all their non canonical functions alter redox state in some way which creates an adaptive microenvironment with enhanced motility, invasion, metabolism and therapy resistance (Bailleul & Vlashi, 2023).

Understanding how these enzymes function across together provides a foundation for identifying new therapeutic vulnerabilities in glioblastoma.

4.6: Therapeutic Targeting

Given the crucial role played by these enzymes in the survival of glioblastomas, therapy that targets these enzymes impairs their ability to perform these non canonical functions that help GBMs adapt to metabolic and genotoxic stress. Inhibiting the non canonical role of these enzymes include specifically targeting their functions as a transcriptional coactivator, redox adaptation and apoptotic suppression. Due to the high malignancy of glioblastomas even after treatment which is the standard of care, looking at a new therapeutic target (these enzymes) is essential in order to better treat GBM.

4.6.1: Targeting PKM2

Shikonin, a natural naphthoquinone pigment derived from the roots of the plant Lithospermum erythrorhizon, is used commonly in traditional Chinese medicine and is known for its anti-inflammatory properties (Guo et al., 2019). Shikonin directly binds to PKM2 leading to both disrupted glycolytic flux and its moonlighting functions as discussed in previous sections (Park et al., 2022). Shikonin binds to the active site of PKM2 and prevents its conversion to its active tetrameric form. This directly reduces glycolytic flux and cuts off the tumours energy supply. It also inhibits PKM2's non canonical role of activating survival pathways (Park et al., 2022). This weakens the GBM as the cells become ready for apoptosis. Resulting from the PKM2 inhibition by Shikonin, GBM cells experience increased levels of reactive oxygen species (ROS) and glutathione depletion (which leads to depolarization of the outer mitochondrial membrane) (Wang et al., 2021). This is a hallmark of the mitochondrial (or intrinsic)

apoptotic pathway. This cascade is heavily linked to the inhibition of PKM2 which normally regulates the redox balance of the cell. As a result of this mitochondrial dysregulation, key apoptotic proteins are modulated (Wang et al., 2021). Bax and p53 are upregulated which are pro apoptotic proteins. Bcl-2 which is an anti apoptotic protein is downregulated. All of these signal the intrinsic apoptotic pathway (C. Chen et al., 2012). These crucial markers are all shown to be downstream of PKM2 inhibition. The blockade of PKM2 also leads to the activation of caspase 9 and caspase-8 both converging at caspase 3/7 to execute late stage apoptosis (J. Yang et al., 2014). Since blocking these caspases reverse the effects of shikonin, they are proven to be crucial to the working of shikonin following the inhibiting of PKM2. Cells undergo late stage apoptosis instead of cell cycle arrest. This indicates that cell death is dependent on the metabolic disruption of PKM2 instead of the classic cell cycle disruption (J. Yang et al., 2014). PKM2 inhibition also triggers autophagy markers in GBM cells. This autophagy is a secondary stress factor that accelerates apoptosis in the absence of PKM2's regulatory metabolic roles (Park et al., 2022).

The therapeutic target here is not PKM2's non canonical function. It in fact targets its metabolic role. However inhibition of this enzyme also prevents it from performing its non canonical function which results in the GBM not being able to adapt to stress. At this weakened state, apoptosis is then induced.

Contrastingly, its non canonical function may also be directly targeted. In U87 GBM cell lines, treatment shikonin reduces levels of phosphorylated beta catenin reducing the binding of PKM2 to beta catenin (F. Zhang et al., 2015). This suppresses the transcription of oncogenes by this complex like CCND1 and MYC that allow GBM cells to survive under stress. It is important to note that this was tried in other GBM cell lines but it did not work in the same way (F. Zhang et al., 2015).

Like Shikonin, more drugs are to be developed that may potentially target non canonical functions of PKM2 directly as to reduce GBM adaptation and survival so that the harsh tumour microenvironment triggers autophagy as the GBM is unable to adapt to stress.

4.6.2: Targeting ENO1

In U87 and U251 glioblastoma cell lines, stable ENO1 knockdown was found to significantly reduce the phosphorylation of P1K/Akt pathway. ENO1 depletion suppressed P13K activation. This led to reduced PIP3 (phosphatidylinositol (3,4,5)- trisphosphate) levels and decreased phosphorylation of Akt at Ser473 and Thr308 both of which are necessary for Akt activation (Song et al., 2014) . Following this, crucial cell cycle regulators (which are downstream targets of this pathway) such as cyclin D1, cyclin E, Rb and NF-KB were downregulated, not allowing for uncontrolled proliferation. Epithelial markers like E-cadherin increase and mesenchymal markers (Vimentin, Snail, N-cadherin and Slug) were suppressed indicating a reversal of EMT (Epithelial to Mesenchymal Transition) and overall loss of invasive potential (Qisheng et al., 2017). Hence, targeting ENO1 was found to directly disrupt the P13K/ Akt pathway and suppress both cell proliferation and invasion (Song et al., 2014).

In numerous glioblastoma cell lines, the molecule miR-22 was significantly downregulated compared to normal astrocytes. miR-22 levels also inversely correlated with the grade of glioma. To investigate this, miR-22 mimics were made and then found to target ENO1. A luciferase assay confirmed that miR-22 directly suppresses ENO1 translation (Ma et al., 2021). With reduced number of ENO1 in GBM cells, GBMs lose all the survival advantages that ENO1 triggers and coordinates under stress making it easier to induce cell death.

Similar to this, for ENO1 many potential treatments have been discovered to knock down ENO1 expression completely instead of targeting each non canonical function (Trejo-Solis et al., 2023). Conversely, unlike ENO1, one of the specific treatments for PKM2 was individually targeting its non canonical function. Investigating the inhibition of each role individually for ENO1 might be a new angle that shows promise instead of complete knockdown which would prevent even the metabolic functioning of cells.

4.6.3 Targeting GAPDH

In C6 glioma models, hypoxia induced stress promotes overexpression and oxidative modification of Hsp-70 to bind to GAPDH preventing aggregation and hence loss of function. AEAC (N-amino-ethyl amino colchicine derivative) when applied disrupted this protective complex formed when Hsp-70 binds to GAPDH. This led to aggregation of GAPDH promoting cytotoxicity and ultimately GBM cell death (Mikeladze et al., 2021).

Additionally, stable knockdown of GAPDH using shRNA (short hairpin RNA) significantly reduces cell proliferation, glioma metabolism and tumorigenicity. GAPDH depletion disrupts glycolytic flux, impairing ATP production and biosynthetic intermediate production. Beyond this, lack of GAPDH caused increased expression of proapoptotic pathways which would otherwise be neutralised by the Hsp-70 - GAPDH complex (Mikeladze et al., 2021).

Under few cases of genotoxic stress where GAPDH is acetylated at Lysine 160 by p300/CBP. This modification increases p53 mediated transcription of pro apoptotic genes like PUMA and Bax (Trejo-Solis et al., 2023). This is a notably unique feature of GAPDH where it can initiate cell death on its own as part of its non canonical function. The natural compound Micheliolide binds GAPDH at Cysteine 247 facilitating its nuclear import and potentiating its acetylation and Lysine 160 (J. Guo et al., 2025). This results in the strong activation of the mitochondrial apoptotic pathway through genes like PUMA and Bax. While GAPDH inhibition shows potential, translating this into therapy remains difficult. GAPDH is expressed in almost all tissues and plays an essential role in glycolysis, so complete inhibition could disrupt normal cellular metabolism. Any effective strategy would therefore need to achieve tumour-specific selectivity and restricted delivery, especially since neurons are also highly glycolytic.
Through different treatments that in some form inhibit the moonlighting function (directly or indirectly) or knock down the enzyme altogether, it is seen that GBM may potentially become more sensitised to stress, which reduces its malignancy. These may be potential targets that will improve GBM prognosis

and survival whether the medication is used individually or in combination with treatments that are standard of care.

FUTURE DIRECTIONS

Despite the developments mentioned earlier, the clinical targeting of ENO1, GAPDH, and PKM2 remains limited by three major challenges: isoform specificity, systemic toxicity, and restricted brain delivery. Each of these glycolytic enzymes has essential housekeeping isoforms required for normal cellular metabolism, making non-selective inhibition potentially lethal (Israelsen & Vander Heiden, 2015). For example, GAPDH and ENO1 are ubiquitously expressed in neurons and glial cells, so broad inhibition would disrupt ATP production in healthy tissue (Israelsen & Vander Heiden, 2015). PKM2, however, is a cancer-associated isoform produced by alternative splicing of the PKM gene, replacing exon 9 (PKM1) with exon 10 (PKM2) (Israelsen & Vander Heiden, 2015). This difference provides a unique molecular signature that can be selectively targeted without affecting normal metabolic function.

For PKM2, PROTACs (proteolysis-targeting chimeras) could in theory be designed to selectively degrade the tumour-specific PKM2 isoform while sparing PKM1, which dominates in normal differentiated tissue. PROTACs are heterobifunctional molecules that recruit an E3 ubiquitin ligase to a target protein and tag it for degradation by the proteasome (Pettersson & Crews, 2019). PKM2's structural distinction, the replacement of exon 9 by exon 10, creates unique surface motifs that could in theory be recognised by a ligand component of a PROTAC molecule to induce ubiquitination and proteasomal degradation specifically in glioma cells (Pettersson & Crews, 2019; Israelsen & Vander Heiden, 2015). This would suppress glycolytic hyperactivation and block PKM2's transcriptional co-activator functions that drive tumour growth (Israelsen & Vander Heiden, 2015).

For ENO1 and GAPDH, which are essential for basal glycolysis in normal tissue, full protein degradation would be unsafe (Israelsen & Vander Heiden, 2015). Instead, CRISPR interference (CRISPRi) or RNA-guided repression systems could be looked into to down-regulate overexpression rather than eliminate it altogether. CRISPRi uses a catalytically inactive Cas9 (dCas9) fused to a transcriptional repressor domain such as KRAB that binds to the promoter region and blocks transcription initiation (Qi et al., 2013). This approach allows partial gene silencing, reducing enzyme abundance only in tumour cells where overexpression drives malignancy (Qi et al., 2013). Such modulation could restore enzyme expression to near-normal levels rather than abolish activity altogether, minimising cytotoxicity to normal tissue (Yeo et al., 2023).

However, neither PROTAC- nor CRISPRi-mediated modulation of ENO1, GAPDH, or PKM2 has yet been successfully demonstrated in glioblastoma models. Key barriers include the difficulty of achieving tumour-specific delivery across the blood–brain barrier, incomplete selectivity for oncogenic isoforms, and the risk of off-target editing or protein degradation. The heterogeneity of GBM tissue and the tumour's capacity for metabolic rewiring also limit the sustained efficacy of single-target approaches. Additionally, both PROTACs and CRISPR-based systems currently face challenges in intracellular stability, immune recognition, and large-scale delivery efficiency (Saraiva et al., 2016).

The blood–brain barrier (BBB) remains a central obstacle for all three enzyme-targeting strategies. The BBB consists of tightly joined endothelial cells that prevent most drugs from entering the brain parenchyma (Saraiva et al., 2016). Delivery systems such as lipid nanoparticles, transferrin receptor targeted vesicles, or adeno-associated viral (AAV) vectors are being tested to transport CRISPR and PROTAC molecules across this barrier (Saraiva et al., 2016). By coupling these systems with tumour-homing ligands such as RGD peptides that bind integrins overexpressed on glioma cells, the therapeutic payload can be directed specifically to the malignant tissue, minimising neurotoxicity (Saraiva et al., 2016).

Although these approaches remain theoretical at present, refining them to achieve tumour-specific delivery and sustained gene or protein modulation could make them valuable adjuncts to current therapies. Once optimised, transient enzyme inactivation could be combined with chemotherapy or radiotherapy to further stress tumour cells that are already metabolically compromised. Such integration could increase treatment sensitivity by targeting the enzymes that enable GBM's adaptive survival mechanisms, offering a future direction for precision therapy.

CONCLUSION

This review focuses on how glioblastomas adapt to cellular stressors and their harsh tumour microenvironment through the non canonical functions of the glycolytic enzymes PKM2, ENO1 and GAPDH. These enzymes that are overexpressed due to the Warburg effect in cancer cells exhibit moonlighting roles after translocating to other intracellular regions, most commonly the nucleus. This is triggered by different oncogenic stimuli. This also aids in transcriptional reprogramming, immune evasion, chromatin remodelling, extracellular matrix degradation and more functions that ultimately help the cell survive.

PKM2 enhances the transcription of oncogenic proteins like c-Myc and cyclin D1 supporting tumour growth and metabolic reprogramming, whereas ENO1 promotes immune evasion and proliferation through the PI3K/Akt pathway. Similarly, GAPDH participates in transcriptional regulation and apoptosis, specifically modulating gene expression through interactions with different transcriptional coactivators and signalling pathways.

The synergistic mechanism of these three enzymes in GBMs helps coordinate multiple hallmarks of cancer such as sustained proliferation, immune evasion and survival under stress. Understanding their role reveals potential therapeutic targets that extend beyond their general glycolytic role. Importantly, this review addresses a key gap in existing literature by integrating the non canonical functions of multiple glycolytic enzymes to explain their collective contribution to GBM stress adaptation. Therapeutic targeting discussed here focuses on strategies that have already been tested, such as Shikonin, miR-22 and AEAC, which act by inhibiting or knocking down these enzymes as a whole. However, future approaches that could show promise include targeting their non canonical functions individually through isoform-specific degradation using PROTACs or transcriptional suppression using CRISPR interference systems. These methods have not yet been successfully demonstrated in GBM models, possibly due to

limitations in tumour-specific delivery, off-target effects and blood–brain barrier penetration. When refined, such approaches could be combined with existing treatments like chemotherapy or radiotherapy, where enzyme isoform inactivation first weakens tumour adaptability before exposure to genotoxic or metabolic stress, potentially improving prognosis and treatment response.

By consolidating experimental evidence and mechanistic insights from molecular and cellular studies, this review provides a framework for understanding how metabolic enzymes contribute to GBM survival and highlights how precision modulation of their non canonical roles could be an important direction for future research in cancer therapeutics.

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