

REVIEW ARTICLE

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Non-canonical functions of enzymes facilitate cross-talk between cell metabolic and regulatory pathways

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Abstract

The metabolic rewiring that occurs during cell transformation is a hallmark of cancer. It is diverse in different cancers as it reflects different combinations of oncogenic drivers, tumor suppressors, and the microenvironment. Metabolic rewiring is essential to cancer as it enables uncontrolled proliferation and adaptation to the fluctuating availability of nutrients and oxygen caused by poor access to the vasculature due to tumor growth and a foreign microenvironment encountered during metastasis. Increasing evidence now indicates that the metabolic state in cancer cells also plays a causal role in tumor growth and metastasis, for example through the action of oncometabolites, which modulate cell signaling and epigenetic pathways to promote malignancy. In addition to altering the metabolic state in cancer cells, some multifunctional enzymes possess non-metabolic functions that also contribute to cell transformation. Some multifunctional enzymes that are highly expressed in cancer, such as pyruvate kinase M2 (PKM2), have non-canonical functions that are co-opted by oncogenic signaling to drive proliferation and inhibit apoptosis. Other multifunctional enzymes that are frequently downregulated in cancer, such as fructose-bisphosphatase 1 (FBP1), are tumor suppressors, directly opposing mitogenic signaling via their non-canonical functions. In some cases, the enzymatic and non-canonical roles of these enzymes are functionally linked, making the modulation of non-metabolic cellular processes dependent on the metabolic state of the cell.

Introduction

Metabolism is not just a permissive process that exists exclusively to meet cellular biosynthetic and bioenergetic needs. Instead, metabolism is intricately connected to multiple cellular processes, as certain metabolic intermediates function as cofactors or substrates for the post-translational modification of proteins or the modification of DNA during epigenetic regulation. These metabolic intermediates can become rate-limiting depending on the metabolic state of the cell. Examples of such metabolites include acetyl-CoA (AcCoA), *s*-adenosylmethionine

(SAM), succinate, fumarate, 2-hydroxyglutarate (2HG) and α -ketoglutarate (α KG). The various roles of these molecules, also called oncometabolites, have been studied extensively in the context of cancer¹.

However, not only metabolic intermediates have the ability to couple the metabolic state of the cell to other cellular functions. In addition to their canonical enzymatic function within the metabolic network, various multifunctional (moonlighting) metabolic enzymes perform non-canonical functions in a variety of cellular processes. Among the first multifunctional enzymes to be discovered were the glycolytic enzymes that function as crystallins in the lens of the eye². Since then, it has become clear that the non-canonical functions of metabolic enzymes are quite common. Every single enzyme in the glycolytic cascade and several enzymes from other metabolic pathways have been found to be

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Table 1 The non-canonical roles of metabolic enzymes

Enzyme	Pathway	Non-canonical role	Link	Ref
HK2	Glycolysis	Regulation of mTOR	Yes	74
		Inhibitor of apoptosis	Yes	75
		RNA regulation	Yes	112
PGI	Glycolysis	Extracellular cytokine	No	113
PFK-1	Glycolysis	Transcription	Yes	91
Aldolase	Glycolysis	AMPK regulation	Yes	65
		F-actin binding	Yes	77
		Endocytosis	Yes	114
		Wnt signaling	n.d.	115
		p53 regulation via DNA-PK	n.d.	116
		RNA binding	n.d.	98
		GAPDH	Glycolysis	Regulation of mTOR
RNA binding (T cells)	Yes			102
RNA binding	n.d./No			100
Transcription	Yes			117
Regulation of apoptosis	No			118
Cytoskeleton	No			118
Vesicular trafficking	No			118
PGK	Glycolysis	DNA repair	n.d.	118
		Extracellular disulfide reductase	No	119
		Protein kinase	No	4,5
PGM	Glycolysis	RNA binding	n.d.	98
		Regulator of the cytoskeleton	No	39
		Transcription (Myc)	n.d.	94
ENO1	Glycolysis	Transcription (Foxp3)	Yes	95
		Transcription	Yes	17
PKM2	Glycolysis	Protein kinase	n.d.	17
		Transcription	n.d.	17
		Extracellular (pro-angiogenesis)	No	120
LDH	Glycolysis	Transcription	Yes	121
		RNA binding	n.d.	98
PDH	TCA cycle	Transcription (E2 subunit)	n.d.	122
ACO2	TCA cycle	RNA binding (Iron regulatory protein 1 (IRP1))	Yes	123
MDH1	TCA cycle	Transcription	No	61

Table 1 continued

Enzyme	Pathway	Non-canonical role	Link	Ref
FBP1	Gluconeogenesis	Transcription	No	47
		MAPK signaling	No	48
TS	Thymidylate synthesis	RNA binding	Yes	103
DHFR	Thymidylate synthesis	RNA binding	Yes	103
MVK	Mevalonate pathway	RNA binding	Yes	108
GMPS	Purine synthesis	p53 stabilization	No	59
KHK	Fructose metabolism	Protein kinase	No	124
MTHFD2	Folate metabolism	Nuclear MTHFD2 promotes proliferation independent of enzymatic activity	No	125
		Transcription (with PPARα)	No	35
		Transcription (with PPARα)	No	38
EHHADH	Fatty acid degradation	RNA binding	n.d.	98
		RNA binding	n.d.	98
G6PD	Pentose phosphate pathway	RNA binding	n.d.	98
GDH	Glutamine metabolism	RNA binding	n.d.	98
SHMT	Serine synthesis	RNA binding	n.d.	98

A list of the multifunctional enzymes identified to date in vertebrates, their non-canonical roles, the metabolic pathway they are associated with and whether their canonical and non-canonical functions are linked. In addition to these enzymes, numerous enzymes have been identified as RNA-binding proteins in RNA interactome screens but have yet to be validated. For a full list of these enzymes, please see ref.⁹⁷

HK2 hexokinase 2, PGI phosphoglucoisomerase, PFK-1 phosphofructokinase-1, GAPDH glyceraldehyde-3-phosphate dehydrogenase, PGK phosphoglycerate kinase, PGM phosphoglucomutase, ENO1 enolase 1, PKM2 pyruvate kinase muscle isoform 2, LDH lactate dehydrogenase, PDH pyruvate dehydrogenase, ACO2 acetyl-CoA carboxylase 2, MDH1 malate dehydrogenase 1, FBP1 fructose-1,6-bisphosphatase 1, TS thymidylate synthase, DHFR dihydrofolate reductase, MVK mevalonate kinase, GMPS Guanosine 5'-monophosphate synthase, KHK ketoheptokinase, MTHFD2 methylenetetrahydrofolate dehydrogenase 2, HMGCS2 3-hydroxy-3-methylglutaryl-CoA synthase 2, EHHADH enoyl-CoA hydratase/3-hydroxyacyl CoA dehydrogenase, G6PD glucose-6-phosphate dehydrogenase, GDH glutamate dehydrogenase, SHMT serine hydroxymethyltransferase

multifunctional (Table 1). Moreover, some metabolic enzymes, such as PKM2, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and aldolase, carry out multiple non-canonical functions in cellular processes such as transcription, signaling and cytoskeletal dynamics. In some cases, these secondary functions are entirely independent of the canonical enzymatic role and do not

involve regulatory processes in the cell, as in the case of the glycolytic enzymes functioning as crystallins. However, the non-canonical functions of metabolic enzymes often regulate processes that are highly relevant for cell transformation and cancer development: they promote uncontrolled cell proliferation, induce resistance to apoptosis or enhance cell migration. Other enzymes have non-canonical functions that oppose mitogenic signaling or promote apoptosis under conditions of stress, thus having a tumor suppressive role. In many of these cases, canonical and non-canonical enzyme functions are often interdependent, thus connecting the activity of cancer-relevant cellular processes to the metabolic state of the cell.

Here, we focus on multifunctional enzymes that have been shown to play a non-canonical role in cancer. These functions represent another layer of complexity within the regulatory network in cancer and provide additional challenges for therapeutic targeting.

Oncogenic non-canonical functions of metabolic enzymes

Of the numerous multifunctional enzymes described, several have pro-proliferative and/or anti-apoptotic roles within various non-metabolic cellular processes and can contribute to cell transformation and tumor development. For some of these enzymes, the switch from canonical to non-canonical function is induced by the action of oncogene-activated signaling cascades via post-translational modifications. For others, the non-canonical function is intrinsic to the enzyme and is promoted in cancer by the elevated expression of the enzyme.

Glycolytic enzymes with non-canonical functions as protein kinases in cancer

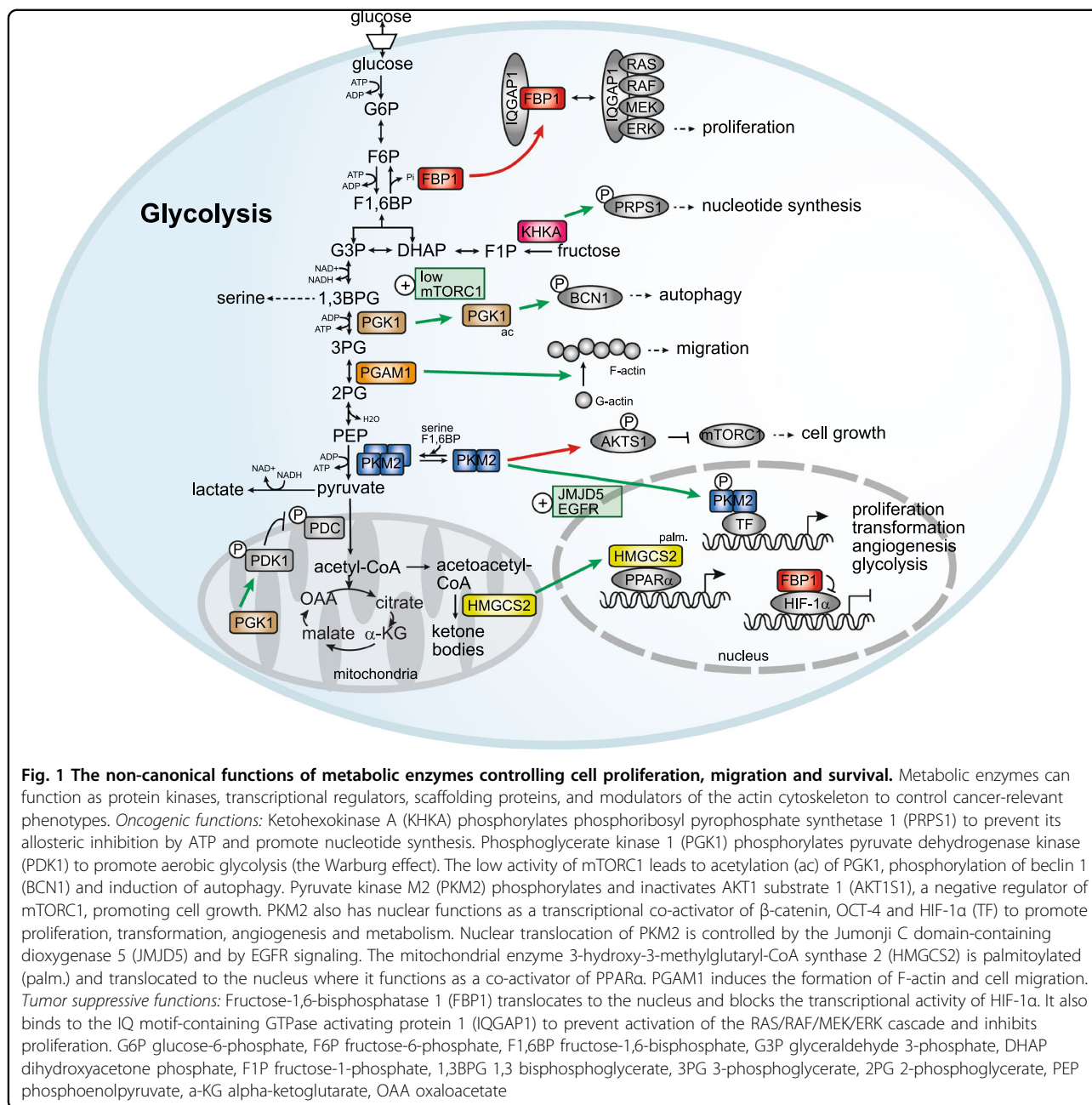
In recent years, several examples of metabolic enzymes acting as phosphate transferases in metabolism but having a secondary role as protein kinases have been uncovered. One of these is ketohexokinase (KHK), the enzyme that converts fructose to fructose-1-phosphate, which subsequently enters glycolysis at the level of aldolase. KHK has a secondary function as a protein kinase³. During the formation of hepatocellular carcinoma (HCC), c-MYC induces an isoform switch from KHKC to KHKA. Interestingly, KHKA, but not KHKC, interacts with and phosphorylates phosphoribosyl pyrophosphate synthetase 1 (PRPS1), a key enzyme in nucleotide synthesis. This phosphorylation prevents the allosteric inhibition of PRPS1 by ADP, resulting in enhanced de novo nucleotide biosynthesis during HCC tumor growth (Fig. 1)³.

Likewise, the glycolytic enzyme phosphoglycerate kinase 1 (PGK1) is also a protein kinase in glioblastoma cells^{4,5}. Upon phosphorylation by ERK1/2, induced by hypoxia or expression of the oncogenes K-RAS (G12V) or

Braf (V600E), PGK1 is transported to the mitochondria where it phosphorylates and activates pyruvate dehydrogenase kinase 1 (PDK1). This, in turn, inhibits the pyruvate dehydrogenase complex (PDC), leading to increased lactate production and glycolytic activity (Fig. 1)⁴. In addition, glutamine deprivation or hypoxia, but not glucose deprivation, can lead to the acetylation of PGK1, which then phosphorylates beclin 1 (BCN1), a scaffolding protein essential for the formation of autophagosomes during autophagy⁵. Acetylation of PGK1 is triggered by the low activity of complex 1 of the mammalian target of rapamycin (mTORC1) upon glutamine starvation or treatment with the mTORC1 inhibitor torin 1 (Fig. 1). Analysis of human tumor samples also indicates a strong correlation between PGK-1 acetylation, BCN1 phosphorylation and poor prognosis in glioblastoma⁵.

Another metabolic enzyme that doubles as a protein kinase is the M2 isoform of the glycolytic enzyme pyruvate kinase (PKM2), which has received much attention in the context of cancer metabolism. This isoform of pyruvate kinase is frequently upregulated in cancer due to a switch in alternative splicing from the PKM1 isoform to PKM2, induced by the transcription factor c-MYC⁶. In cancer, PKM2 plays a key role in fueling anabolic metabolism by diverting metabolic flux from glycolysis into the pentose phosphate pathway (PPP) and the serine synthesis pathway (SSP)⁷. This specific role of PKM2 is mediated by its ability to change its conformation between a catalytically low activity dimer and a high activity tetramer. The balance between these two conformations is determined by different allosteric regulators, as well as by intracellular signaling, mostly via post-translational modifications (PTM). The tetrameric form of PKM2 is stabilized by fructose-1,6-bisphosphate (F1,6BP), the product of phosphofructokinase 1 (PFK1)⁸. This mechanism of allosteric feed-forward regulation couples the last reaction of glycolysis with the first rate-limiting step. Another allosteric activator of PKM2 is serine⁹, which is mostly taken up by cells but can also be synthesized from 3-phosphoglycerate (3PG), an intermediate of glycolysis. When serine or F1,6BP levels are low, allosteric activation of PKM2 is reduced and glycolytic metabolites can be rerouted into the pentose phosphate and serine synthesis pathways. Once the levels of serine and F1,6BP increase again, PKM2 forms a tetramer and catalyzes the rapid formation of pyruvate, thereby removing glycolytic intermediates. PKM2 can dynamically shift (or oscillate) between its dimeric and tetrameric form in response to alterations in the levels of its allosteric regulators. In fact, this feature of PKM2 was used to generate a FRET biosensor to monitor oscillatory glycolytic activity in pancreatic β -cells¹⁰.

Many of the PTMs induced by intracellular signaling promote the dimeric form of PKM2 and do so by interfering with F1,6BP binding. The phosphorylation of



fibroblast growth factor receptor type 1 (FGFR1) at Y105 releases F1,6BP from PKM2 and promotes dimerization¹¹. Interestingly, this has been suggested to be a “transprotein” mechanism dependent on the K433 residue in PKM2 that directly interacts with the F1,6BP molecule. Specifically, a modified PKM2 molecule induces the release of F1,6BP from other, unmodified PKM2 molecules by interfering with the interaction of K433 and F1,6BP¹¹. Moreover, this ability to induce the release of F1,6BP from other PKM2 molecules is not unique to tyrosine-phosphorylated PKM2. Different phosphotyrosine peptides with an Src kinase-like motif can induce the release

of F1,6BP from PKM2¹², indicating that various tyrosine-phosphorylated proteins can influence PKM2 oligomerization. In addition to phosphorylation, acetylation at the K433 residue by P300 acetyltransferase and sumoylation by the SUMO-E3 ligase PIAS3, prevent F1,6BP from binding to PKM2, leading to the dimerization and nuclear translocation of PKM2^{13,14}. The ability of PKM2 to block glycolysis at the level of pyruvate kinase seems to be essential for cancer development, as cancer cells expressing only the PKM1 isoform have a reduced tumor-forming capacity¹⁵. Nevertheless, some non-proliferating populations of cancer cells within a tumor may depend on

PKM1 expression, demonstrating the complexity of metabolic regulation in cancer¹⁶.

The enzymatically inactive dimeric form of PKM2 is widely reported to have non-canonical functions as either a protein kinase or as a transcriptional co-activator (Fig. 1). Numerous proteins have been identified and studied as targets of PKM2 protein kinase activity, including histone H3, the transcription factor STAT3, the spindle checkpoint protein Bub3 (reviewed in refs.^{17,18}) and the apoptosis regulator Bcl-2¹⁹. A recent screen for PKM2 substrates identified over 400 proteins as potential targets of PKM2 protein kinase activity. The same study also demonstrated that AKT1 substrate 1 (AKT1S1), a negative regulator of mTORC1, is negatively regulated by PKM2-dependent phosphorylation in renal cancer²⁰. The effects of the PKM2 kinase function are generally pro-mitogenic or anti-apoptotic and are frequently induced by post-translational modifications of PKM2, including ERK1/2 or Aurora B dependent phosphorylation (reviewed in refs.^{17,18}). In contrast to KHKA and PGK1, which use ATP as a phosphate donor for protein phosphorylation, PKM2 has been reported to use its glycolytic substrate, phosphoenolpyruvate (PEP), as a phosphate donor for its non-canonical function as a protein kinase^{19–23}. Nevertheless, the protein kinase function of PKM2 has been called into question by in vitro experiments that failed to find evidence for PEP- or ATP-dependent kinase activity²⁴.

Metabolic enzymes as transcriptional regulators

In addition to its non-canonical role as a protein kinase, PKM2 has also been reported to act as a transcriptional co-activator in the nucleus (Fig. 1). PKM2 interacts with the transcription factor OCT-4 in embryonic carcinoma cells and glioma spheroids and modulates the expression of OCT-4 target genes^{25,26}. In renal cell carcinoma (RCC) and breast cancer cells (MCF7), PKM2 interacts with Hif-1 α and promotes its transcriptional activity^{27,28}. Interestingly, in MCF7 cells, Jumonji C domain-containing dioxygenase 5 (JMJD5) is required for PKM2 nuclear translocation and Hif-1 α activation; JMJD5 interacts with the dimeric form of PKM2 and prevents its re-tetramerization²⁸. The role of PKM2 as co-activator of Hif-1 α is not limited to cancer, as nuclear localization of PKM2 and subsequent activation of Hif-1 α transcription has also been observed during macrophage activation²⁹. In this context, the PKM2 nuclear translocation and activation of Hif-1 α can be inhibited by agents that promote the tetramerization and activation of PKM2²⁹. In glioblastoma cells, activation of the epidermal growth factor receptor (EGFR) leads to phosphorylation of PKM2 and β -catenin by ERK1/2 and c-Src kinases, respectively, and subsequent nuclear localization of both proteins^{30,31}. In the nucleus, PKM2 promotes the transcriptional

activity of β -catenin both as a co-activator and as a protein kinase, by phosphorylating histone-H3, which leads to its acetylation^{22,31}.

Other metabolic enzymes also act in transcription within the context of cancer. A recent example of this is the regulation of the peroxisome proliferator-activated receptor α (PPAR α) by the enzyme 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2), the rate-limiting enzyme in ketogenesis, in hepatic tissue³². HMGCS2, as well as other enzymes of the ketone synthesis pathway, are upregulated in some cancers, including androgen-independent prostate cancer^{33,34}, colorectal carcinoma (CRC) and oral squamous cell carcinoma (OSCC)³⁵. HMGCS2 translocates to the nucleus and, through a process that requires HMGCS2 palmitoylation, acts as a co-transactivator for PPAR α to regulate its own expression (Fig. 1)^{36,37}. In CRC and OSCC cells, enhanced invasion and migration induced by the increased expression of HMGCS2 is independent of its catalytic activity but requires interaction with PPAR α to promote expression of the tyrosine kinase Src^{35,36}. In addition, the bifunctional peroxisomal enzyme enoyl-CoA-hydratase/3-hydroxyacyl-CoA-dehydrogenase (EHHADH) has also been reported to regulate its own expression via its interaction with PPAR α ³⁸.

PGAM1 as a modulator of the cytoskeleton

Phosphoglycerate mutase 1 (PGAM1), the glycolytic enzyme responsible for the conversion of 3PG to 2-phosphoglycerate (2PG), is upregulated in a variety of cancers³⁹ and is associated with enhanced cell migration and motility^{40,41}. This effect was attributed to the non-canonical function of PGAM1 as a modulator of the cytoskeleton. A screen in HEK293 cells identified the G-actin isoform of alpha smooth muscle actin (ACTA2), which is associated with metastatic potential in lung adenocarcinoma⁴², as a novel interaction partner for PGAM1. The interaction between PGAM1 and ACTA2 is essential for cell migration and cytoskeleton remodeling in a breast cancer cell line, and depletion of either PGAM1 or ACTA2 inhibits cell motility by reducing F-actin formation (Fig. 1). Expression of a mutated form of PGAM1 that is unable to bind ACTA2 but retains its enzymatic activity did not restore F-actin assembly and cell migration. Conversely, deletion of the catalytic activity or pharmacological inhibition of PGAM1 did not interfere with its ability to modulate cytoskeletal dynamics³⁹, confirming that its canonical activity is dispensable in this context. In addition, depletion of PGAM1 in an in vivo model of breast cancer significantly reduced the number of lung metastases³⁹ and co-expression of ACTA2 and PGAM1 correlated with poor prognosis in human breast cancer patients.

Non-canonical, tumor suppressive functions of metabolic enzymes

To date, a few enzymes have been found to act as tumor suppressors; well-characterized examples are the TCA cycle enzymes succinate dehydrogenase (SDH) and fumarate hydratase (FH). Loss of function mutations in the genes coding for SDH and FH can lead to cancer, which can be explained by the fact that succinate and fumarate, the substrates for SDH and FH, inhibit the prolyl hydroxylase (PHD) family of enzymes. Inhibition of PHDs leads to the normoxic stabilization of HIF1 α , as it is hydroxylated and targeted for degradation by PHDs⁴³. Both SDH and FH act as tumor suppressors via their canonical functions, however, several enzymes have been discovered that possess non-canonical tumor suppressive functions; they either directly oppose mitogenic signaling or promote apoptosis and cell cycle arrest under conditions of stress.

FBPase1 is a tumor suppressor that counteracts the HIF-1 α and MAPK/ERK pathways

Fructose-1,6-bisphosphatase 1 (FBP1) is the rate-limiting enzyme in gluconeogenesis and converts F1,6BP to F6P. FBP1 has non-canonical functions that oppose cell transformation. The expression of FBP1 is reduced in several cancer types, including HCC^{44,45}, colon and breast cancers⁴⁶, renal carcinoma⁴⁷ and pancreatic cancer^{48,49}. Moreover, processes that promote tumorigenesis also repress FBP1 expression^{44–46,49,50}. In ccRCC, FBP1 interacts with HIF-1 α on promoters that contain hypoxia response elements (HRE) to repress the transcription of HIF-1 α target genes (Fig. 1)⁴⁷. Ectopic expression of FBP1 in ccRCC cells reduced their glycolytic activity and NADPH production and blocked their ability to form xenograft tumors. Interestingly, the tumor suppressive activity of FBP1 is primarily mediated by its non-canonical nuclear functions; a reduction in tumor growth was still observed after the expression of a catalytically inactive mutant of FBP1, while the addition of a nuclear export signal (NES) completely abolished the effect⁴⁷. Compared with the wild-type protein, catalytically inactive FBP1 had a reduced ability to suppress ccRCC cell proliferation under low glucose conditions, which suggests that enhancing gluconeogenesis also contributes to tumor suppression by FBP1⁴⁷.

Another non-canonical tumor-suppressing function of FBP1 has been identified in pancreatic cancer. Here, FBP1 interacts with the IQ motif containing GTPase activating protein 1 (IQGAP1), a scaffolding protein that is essential for the RAS/RAF/MEK/ERK signaling cascade that promotes RAS-driven tumorigenesis (Fig. 1)⁵¹. Modulation of ERK1/2 phosphorylation by FBP1 overexpression or knock-down is dependent on the presence of IQGAP1 and ERK1/2 and FBP1 compete for binding

to its WW domain. Catalytically inactive FBP1, as well as a 45 AA fragment containing the IQGAP1 binding domain of FBP1, were sufficient to block ERK1/2 phosphorylation, inhibit proliferation and the formation of xenograft tumors in PDAC cell lines. However, the catalytic activity of FBP1 also contributes to tumor suppression in this system, as the wild-type protein, which retains its ability to reduce glucose uptake and lactate secretion, had a stronger effect on proliferation and tumor formation compared with the deletion mutants⁴⁸.

GMPS and MDH1 promote p53 function under conditions of stress

Guanosine 5'-monophosphate synthase (GMPS) catalyzes the glutamine-dependent conversion of xanthosine 5'-monophosphate (XMP) to guanosine 5'-monophosphate (GMP), which is the last reaction in the de novo synthesis of guanine nucleotides. GMPS interacts with the deubiquitylation enzyme USP7 (also called HAUSP)^{52,53}. GMPS is an allosteric activator of USP7 and stabilizes its active state resulting in hyperactivation of USP7⁵⁴. The interaction between GMPS and USP7 promotes the deubiquitylation of H2B and modulates the expression of ecdysteroid target genes⁵⁵ and homeotic genes⁵² in *Drosophila*, as well as expression of the latent origin of replication of the Epstein-Barr virus in HeLa cells^{53,56}.

Interestingly, USP7 can also be found in a complex with the tumor suppressor p53 and its negative regulator MDM2, an E3 ubiquitin ligase that targets p53 for degradation but can also act as transcriptional co-repressor for p53⁵⁷. In unstressed cells, USP7 deubiquitylates both p53 and MDM2 and contributes to a finely balanced state in which p53 is continuously degraded⁵⁸. The interaction between MDM2 and p53 is disrupted upon cellular stress, leading to the stabilization of p53⁵⁷. Knock-down of GMPS prevents p53 stabilization in response to genotoxic stress⁵⁹, while concomitant overexpression of both USP7 and GMPS leads to p53 stabilization and apoptosis, even in the absence of genotoxic stress⁵⁹. Mechanistically, this can be explained by a change in complex formation, with GMPS replacing MDM2 and the resulting complex between USP7, GMPS and p53 stabilizes p53 (Fig. 2)⁵⁹. In unstressed cells, GMPS itself is ubiquitylated by the E3 ligase TRIM21 and sequestered in the cytoplasm. In contrast, genotoxic stress induces GMPS deubiquitylation by USP7, allowing for its nuclear translocation. Interestingly, the enzymatic activity of GMPS is not needed for this non-canonical function; a catalytically inactive form of GMPS stabilizes p53 to the same extent as the wild-type protein when overexpressed with USP7⁵⁹. While it is clear that GMPS is primed to respond to genotoxic stress by activating USP7, it is unclear how genotoxic stress triggers USP7 activation in the first place. Interestingly, inhibition of inosine-5'-

monophosphate dehydrogenase (IMPDH), the enzyme directly upstream of GMPS, leads to the nuclear translocation of GMPS and the stabilization of p53, indicating that the trigger for GMPS may be disrupted nucleotide biosynthesis. Remarkably, it has been reported that upon replicative stress and during the G2 phase of the cell cycle, IMPDH itself translocates to the nucleus, where it represses the expression of histones and the master transcriptional regulator of G1/S transition, E2F⁶⁰. These findings show that GMPS and IMPDH, two enzymes that play a key role in supporting proliferation through their canonical functions in de novo purine synthesis, also play a role in opposing proliferation through their non-canonical functions. It will be interesting to determine, in future studies, whether these functions are interconnected.

Malate dehydrogenase 1 (MDH1), a cytoplasmic enzyme that catalyzes the conversion of malate to oxaloacetate as part of the malate/aspartate shuttle, also regulates p53. MDH1 was identified in a screen of enzymes in central carbohydrate metabolism for p53 interaction partners⁶¹. Upon glucose starvation, both p53 and MDH1 are translocated to the nucleus where MDH1 acts as a transcriptional co-activator of p53 target genes by binding to p53-response elements in the promoters of p53 target genes (Fig. 2)⁶¹. Interestingly, while MDH1 does not interfere with the p53/MDM2 interaction, overexpression and depletion experiments show that MDH1 inhibits p53 ubiquitylation and prevents MDM2-dependent cytoplasmic retention of p53, thereby promoting apoptosis in response to glucose starvation⁶¹. As in the case of GMPS, the catalytic activity of MDH1 is not needed for this non-canonical function⁶¹.

Non-canonical enzyme functions mediate cross-talk between metabolism and signaling in cancer

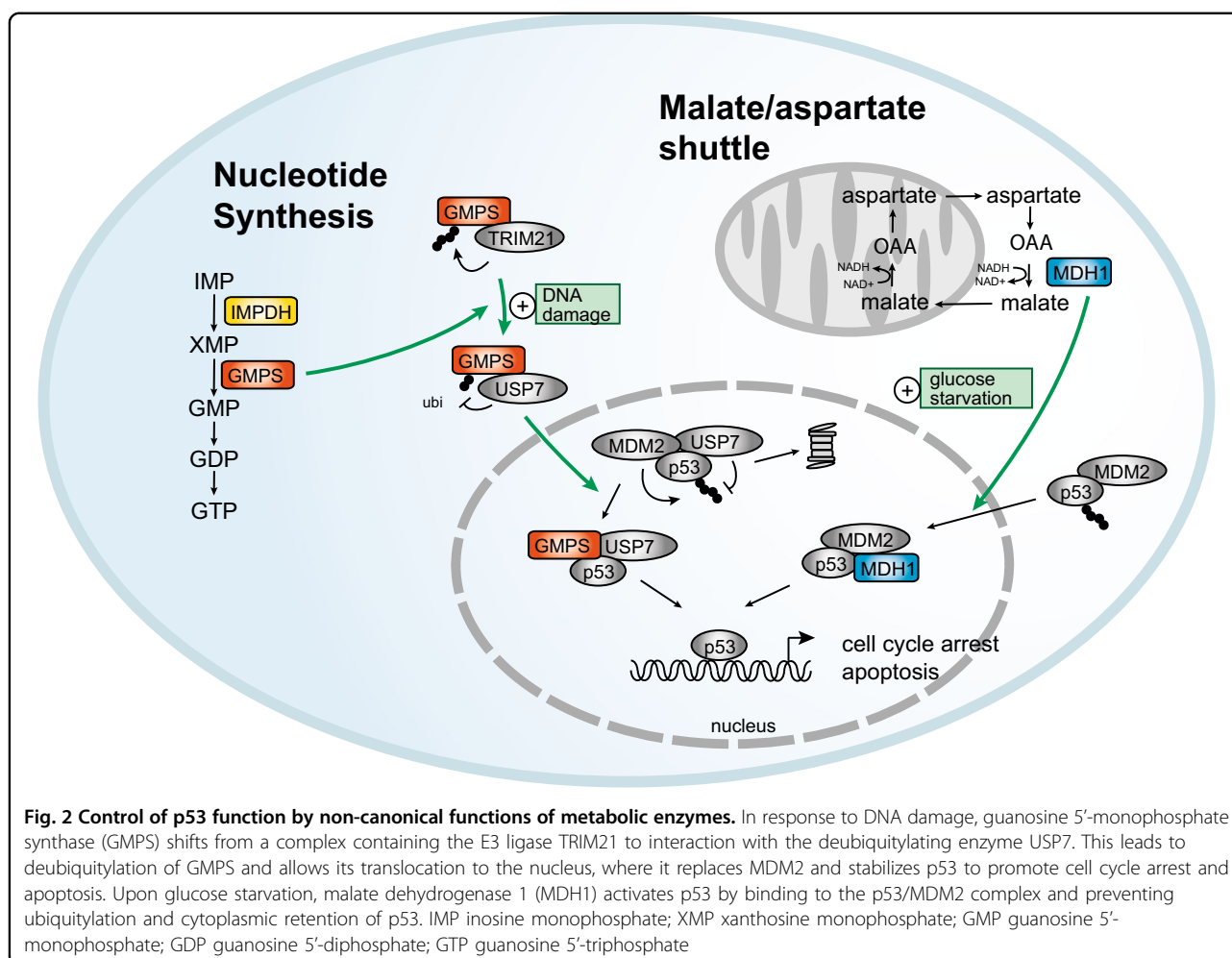
In some cases, the non-canonical functions of metabolic enzymes are intricately linked to their canonical activity; the two functions can be dependent on each other or even be mutually exclusive. Such enzymes can act as sensors that modulate non-metabolic functions in a manner dependent on the metabolic state of a cell. Most of the enzymes that fit this definition belong to the glycolytic pathway. By switching between canonical and non-canonical activities, these enzymes can promote proliferation when glycolytic activity is high but block it when glycolysis is reduced, such as under conditions of nutrient starvation.

Regulation of AMPK and mTORC1 by glycolytic activity

The mammalian target of rapamycin complex 1 (mTORC1) is a serine/threonine kinase and part of a major nutrient-sensing pathway. It integrates information

on nutritional availability in the cell (i.e., amino acids, glucose, and oxygen) and growth factor signaling coming from various upstream pathways, including some that are frequently mutated and activated in cancer, such as the PI3K/Akt and the RAS/RAF/MEK/ERK pathways. The net effect of the mTORC1 pathway is to promote growth and anabolic processes (i.e., amino acid, nucleotide, and lipid synthesis) but inhibit catabolic and/or conserving processes such as autophagy⁶². A major regulator of mTORC1 activity is the AMP-activated protein kinase (AMPK), which inhibits mTORC1 directly and indirectly via activation of the mTORC1 inhibitor tuberous sclerosis complex (TSC). The primary function of AMPK is to respond to altered adenosine nucleotide ratios (AMP/ATP or ADP/ATP) by inhibiting anabolic processes and promoting the generation of energy through catabolic reactions. In addition to mTORC1, AMPK targets various other enzymes including acetyl-CoA carboxylase, the rate-limiting enzyme for fatty acid synthesis⁶³. AMPK is activated by phosphorylation at threonine 172, primarily delivered by the liver kinase B1 (LKB1), and is deactivated by dephosphorylation of threonine 172 by several phosphatases. The binding of AMP and ADP to the gamma subunit of the AMPK complex interferes with dephosphorylation and promotes phosphorylation, resulting in the activation of AMPK⁶⁴. In addition, AMP also activates AMPK through allosteric mechanisms⁶⁴.

Apart from this established mechanism of AMPK regulation, aldolase activates AMPK via a non-canonical function even in the absence of an altered adenosine nucleotide ratio⁶⁵. When cells are starved of glucose, AMPK binds to axin and LKB1, forming a complex on the lysosomal membrane with Ragulator, a protein complex involved in nutrient sensing by mTORC1 and the vacuolar ATPase (v-ATPase)^{66,67}. Interestingly, formation of this complex is mediated by the dissociation of aldolase from the lysosomal membrane, where it can also interact with the v-ATPase. All three mammalian paralogs of aldolase have been found to interact with v-ATPase, and in yeast, this interaction depends on the presence of glucose^{68,69}. The trigger for the removal of aldolase from the lysosomal membrane is a reduction in intracellular levels of F1,6BP, the substrate for aldolase in glycolysis, caused by diminished glycolytic activity. As the lysosomal complex is formed, AMPK is phosphorylated and activated by LKB1, which, in turn, phosphorylates its targets, including raptor and TSC2, leading to inhibition of mTORC1 (Fig. 3). In mouse embryo fibroblasts (MEFs), a reduction in intracellular glucose levels leads to a rapid increase in AMPK activity prior to any significant change in AMP/ATP or ADP/ATP ratios⁶⁵. Likewise, in the livers of mice starved for 16 h, which causes a large drop in blood glucose levels, AMPK activity, but not nucleotide ratios, is elevated⁶⁵. The inhibition of mitochondrial



activity or the combined starvation of glucose and glutamine in MEFs did increase the nucleotide ratios, as well as AMPK activity. However, this is a delayed response as opposed to the acute increase in AMPK activity observed upon glucose starvation in the absence of altered nucleotide ratios. Hence, this suggests that at least two different mechanisms exist by which AMPK senses energetic stress, an acute response, mediated by the non-canonical function of aldolase, and a delayed response, dependent on altered nucleotide ratios.

Until recently, it was believed that the inhibition of mTORC1 signaling observed under glucose starvation is mediated exclusively by AMPK. However, this inhibition can occur not only via AMPK, independent of an altered AMP/ATP ratio (as discussed above) but also entirely independent of AMPK. The two mechanisms identified to date that mediate the glucose starvation-induced inhibition of mTORC1 rely on the non-canonical activities of the glycolytic enzymes HK2 and GAPDH (Fig. 3).

In MEFs deficient for both the $\alpha 1$ and $\alpha 2$ subunits of AMPK, glucose starvation can still inhibit mTORC1 activity^{70,71}, suggesting that an AMPK independent mechanism of glucose sensing must exist. Indeed, it was found that GAPDH binds to the GTPase Rheb and prevents its interaction with mTORC1, similar to the inhibition of mTORC1 by TSC1/2^{72,73}. Interestingly, the interaction between GAPDH and Rheb appears to be regulated by glycolytic activity, as it is reduced during culture in high glucose media compared with low glucose media (Fig. 3)⁷². In addition, cells cultured in the presence of the hexokinase inhibitor 2-deoxyglucose (2-DG) strengthened the interaction between GAPDH and Rheb, while the addition of the GAPDH substrate glyceraldehyde-3-phosphate (G3P) prevented it. Importantly, these effects were also found in AMPK $\alpha 1$ -silenced and in TSC1-deficient or TSC2-deficient cells^{72,73}, confirming that this mode of mTORC1 regulation is independent of AMPK and TSC1/2.

The second glycolytic enzyme that regulates mTORC1 activity is hexokinase 2 (HK2). HK2 interacts directly with and inhibits mTORC1 in cardiomyocytes and HEK293 cells during glucose starvation, leading to the activation of autophagy (Fig. 3)⁷⁴. While the kinase domain of HK2 is dispensable for binding to mTORC1, the interaction between the two proteins is inhibited by glucose-6-phosphate (G6P), the product and allosteric inhibitor of HK2⁷⁴. This mode of regulation is reminiscent of the association of HK2 to with the mitochondria as part of its anti-apoptotic role, which is also regulated by G6P (reviewed in ref.⁷⁵). However, these two non-canonical roles of HK2 seem to be independent of each other. The HK2-mTOR interaction is dependent on an mTOR signaling (TOS) motif in HK2 (also found in the mTORC1 protein raptor), whereas an HK2 mutant unable to bind the mitochondria, maintains its ability to inhibit mTOR.

The main advantages of this direct modulation of mTORC1 activity by glycolytic flux, as opposed to AMPK signaling, are likely increased speed and specificity of the response. In addition to the energetic state of the cell, AMPK activity is modulated by various cellular signaling pathways⁷⁶. The induction of biosynthetic processes downstream of mTORC1 requires a sufficient supply of nutrients, including glucose. A direct coupling between mTORC1 activity and glycolytic flux likely provides a safety mechanism that only permits macromolecule assembly when nutrient supplies are adequate. This mode of regulation is also more acute compared with the control by AMPK activity, which is primarily mediated by the cellular energy load. Indeed, GAPDH binding to Rheb occurs within minutes of a reduction in glucose⁷², resulting in a rapid shut down of biosynthetic processes when nutrients are scarce.

Regulation of cytoskeletal dynamics by aldolase and PI3K/Rac signaling

All three paralogs of aldolase bind F-actin to a different degree, with ALDOA having a much higher affinity for actin than ALDOB or ALDOC⁷⁷. The binding of ALDOA to actin is regulated by the presence of its substrate F1,6BP, which reversibly inhibits the ALDOA-actin interaction (Fig. 3)⁷⁷⁻⁷⁹. The interaction of aldolase with actin may also be functionally important as each aldolase tetramer is able to bind multiple F-actin monomers and crosslink actin filaments into rafts *in vitro*^{78,80,81}. In addition, aldolase interacts with the Wiskott-Aldrich syndrome protein (WASp), an actin regulator, via its catalytic domain, and this interaction is reversibly inhibited by F1,6BP^{82,83}.

The mobilization of ALDOA from the cytoskeleton is regulated by PI3K signaling⁸⁴. ALDOA mobilization is essential for the induction of glycolytic activity and is dependent on the activation of Rac, downstream of PI3K,

but independent of the Akt/mTORC1 axis. Inhibition of PI3K leads to a rapid and sustained decrease in glycolytic activity, whereas inhibition of Akt or mTOR only leads to a transient and less pronounced reduction in glycolytic activity. Likewise, the mobilization of glycolytic reserves (the rapid increase in glycolytic activity caused by blocking ATP-dependent respiration using oligomycin) is strongly blunted by the inhibition of PI3K, but not by the inhibition of the Akt/mTOR axis. The inhibition of the mobilization of the glycolytic reserve by inhibiting PI3K is abolished in cells expressing mutant forms of ALDOA that are incapable of binding to actin⁸⁴. Interestingly, in an *in vivo* mouse model of breast cancer, PI3K inhibition only resulted in a modest decrease in glucose uptake, as determined by PET imaging of FDG uptake. However, the conversion of pyruvate to lactate, determined using MRI and hyperpolarized ¹³C-pyruvate, was drastically reduced⁸⁴. *In vivo* ¹³C-glucose tracing in tumors confirms that PI3K inhibition blocks the glycolytic pathway at the level of ALDOA⁸⁴.

These findings indicate that aldolase links glycolytic activity to cytoskeletal dynamics and cell signaling. Under conditions of low glucose availability, ALDOA is bound to the actin cytoskeleton, where it performs its non-canonical role in cytoskeletal organization. When glucose becomes available and the F1,6BP level increases, the interaction between ALDOA and the cytoskeleton is disrupted and the enzyme performs its canonical function in glycolytic metabolism. However, in response to elevated F1,6BP levels, the activation of PI3K/Rac signaling is likely needed to enable the mobilization of ALDOA from the cytoskeleton, via an as-yet-unknown mechanism (possibly involving post-translational modification). Hence, the non-canonical function of ALDOA may have a dual role, linking glycolytic activity with cytoskeletal dynamics and allowing for the rapid elevation of glycolytic activity in response to PI3K signaling. This could be particularly important in cancer cells where PI3K signaling is continuously active.

The YAP/TAZ complex senses glycolytic activity via PFK1

YAP/TAZ are transcriptional coactivators that interact primarily with the TEA domain (TEAD) family of transcription factors to regulate the transcription of genes involved in proliferation and survival⁸⁵. The HIPPO pathway is the best-characterized upstream regulator of YAP/TAZ but they are also influenced by the TGF β , BMP, and Wnt signaling pathways⁸⁵. In addition, YAP/TAZ play an important role in mechanotransduction and are directly regulated by the extracellular matrix (ECM) and the cytoskeleton⁸⁵. Upregulation of YAP/TAZ activity is frequently found in cancer and is believed to be important for resistance to anoikis, the epithelial to mesenchymal transition (EMT) and cell migration^{85,86}.

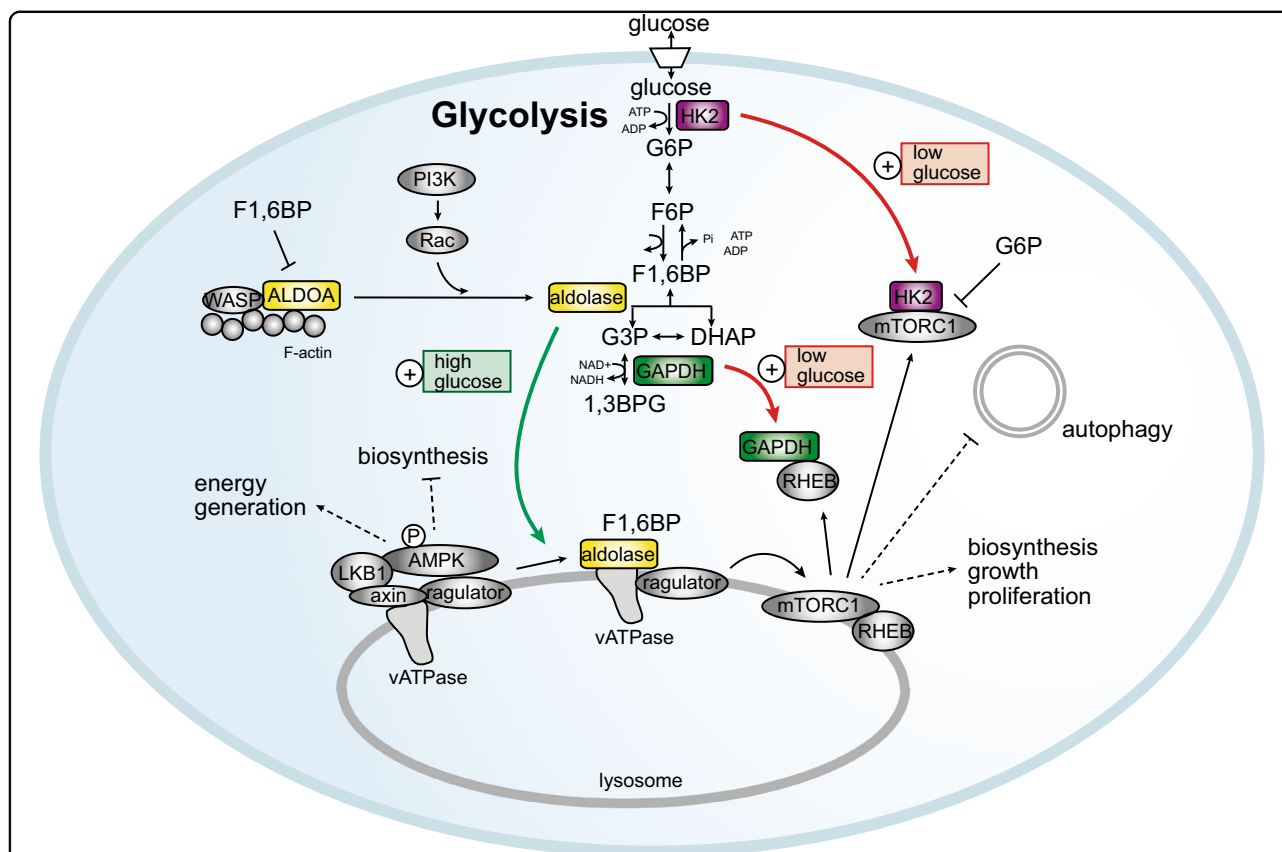


Fig. 3 Cross-talk between metabolism and signaling in cancer. Regulation of AMPK and mTORC1 by glycolytic flux: In the presence of fructose-1,6-bisphosphate (F1,6BP), aldolase interacts with the lysosomal v-ATPase and Ragulator, a component of the mTORC1 nutrient sensing machinery. Upon glucose starvation, F1,6BP levels are reduced and aldolase is removed from this complex, which allows for the association of the v-ATPase-Ragulator complex with AMPK, axin and LKB1, thereby promoting the phosphorylation and activation of AMPK. This mode of regulation contributes to the activation of AMPK by metabolic stress and leads to the inhibition of biosynthesis and the activation of energy-producing processes. Low glucose also promotes the binding of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to the Ras homolog mTORC1-binding protein (RHEB), resulting in the inactivation of mTORC1. Hexokinase 2 can bind directly to mTORC1 during glucose starvation to block macromolecule biosynthesis during growth and proliferation. Glucose-6-phosphate (G6P), the product of the reaction catalyzed by HK2, prevents this interaction, providing an additional mechanism of nutrient sensing and regulation of mTORC1. *Cross-talk between glycolysis, cytoskeletal dynamics and cancer signaling:* Aldolase A (ALDOA) has a non-canonical function in regulating the actin cytoskeleton through binding to the Wiskott Aldrich Syndrome protein (WASP) and F-actin. F1,6BP reversibly inhibits this interaction, thereby providing cross-talk between cellular metabolism and cytoskeletal dynamics. The activation of PI3K/Rac signaling in cancer also leads to the release of ALDOA from the actin cytoskeleton to promote glycolytic activity

A recently discovered additional player in YAP/TAZ regulation is AMPK, which phosphorylates components of the HIPPO pathway, as well as YAP itself in response to glucose starvation, leading to inhibition of YAP/TAZ-dependent transcription^{87–89}. Similar to the regulation of mTORC1 by glycolytic activity via GAPDH and HK2 (discussed above), the activity of YAP/TAZ is also modulated by glycolytic flux via phosphofructokinase 1 (PFK1) (Fig. 4). PFK1 catalyzes the irreversible conversion of F6P to F1,6BP in glycolysis, thereby controlling a key regulatory node that determines the entry of metabolites into glycolysis and the associated PPP and the hexosamine pathways⁹⁰. In breast cancer and mammary epithelial cell lines, YAP/TAZ activity has been found to correlate with

glycolytic activity independent of AMPK⁹¹. This regulation of YAP/TAZ activity is mediated by PFK1, which binds to TEAD1 and 4 in the nucleus. This interaction is required for the stability of the YAP/TAZ/TEAD complex, as depletion of PFK1 leads to its dissociation. Likewise, glucose starvation or inhibition of glycolysis using 2DG inhibits the interaction of PFK1 with TEAD and the interaction of TEAD with YAP/TAZ⁹¹. Furthermore, overexpression of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3), which produces F2,6BP, a potent allosteric activator of PFK1, promotes YAP/TAZ-dependent transcription, whereas a mutant PFK1 that is unable to bind F2,6BP has a low affinity for TEAD⁹¹. These data strongly suggest that YAP/TAZ-dependent

transcription is regulated by glycolytic activity via a non-canonical function of PFK1. The regulation of YAP/TAZ by glycolysis may be of particular significance in breast cancer, as analysis of the expression data from breast tumors revealed a strong correlation between a glycolysis-dependent signature (i.e., genes downregulated by 2DG treatment in breast cancer cell lines) and genes upregulated by YAP/TAZ⁹¹. In addition, genes that are common to both the glycolysis-dependent and the YAP/TAZ signature are highly expressed in high-grade tumors, while expression of the remaining genes from both signatures has no prognostic value⁹¹. As the YAP/TAZ coactivators have also been associated with the induction of stem cell-related properties⁹², the modulation of their activity by glycolytic flux in cancer can affect treatment resistance and disease recurrence.

Enolase1/MPB1 is a transcriptional repressor of Myc

Enolase 1 (ENO1, also known as α -enolase) catalyzes the conversion of 3PG to PEP in glycolysis. However, an alternate translation product of the *ENO1* mRNA has been identified as Myc promoter binding protein 1 (MBP1)⁹³. MBP1 binds to the c-MYC promoter and represses transcription (Fig. 4)⁹³. Both full-length ENO1 and MBP1 localize to the nucleus in leukemia cells, where they interact with the Notch1 intracellular domain (NICD) and suppress transcription of c-MYC and other Notch1 target genes⁹⁴. In addition, overexpression of MBP1 or ENO1 suppresses NICD-induced soft agar colony formation in leukemia cells⁹⁴. The regulation of this non-canonical function of ENO1/MBP1 by glycolytic activity was not assessed in these studies. However, an investigation into the role of metabolism in the differentiation of conventional T cells (T_{conv} cells) into regulatory T cells (T_{reg} cells) in response to T cell antigen receptor (TCR) stimulation, revealed that elevated glycolysis promotes T_{reg} development by modulating the activity of ENO1/MPB1 as a transcriptional repressor (Fig. 4)⁹⁵. The development of T_{reg} cells is accompanied by an increase in glycolytic activity and increased expression of the transcription factor forkhead box P3 (FOXP3) and its splicing variant FOXP3-E2. Inhibition of glycolysis by 2DG during the development of T_{reg} cells attenuates FOXP3/FOXP3-E2 expression and impairs T_{reg} cell function⁹⁵. Interestingly, compared with untreated cells, 2DG-treated T_{reg} cells display elevated nuclear localization of both MBP1 and ENO1, as well as increased recruitment of ENO1 to the FOXP3 promoter. In addition, the effect of 2DG on FOXP3/FOXP3-E2 expression is rescued by silencing ENO1⁹⁵. These data suggest that, at least in T cells, the non-canonical function of ENO1 as a transcriptional repressor and its canonical function as a glycolytic enzyme may be mutually

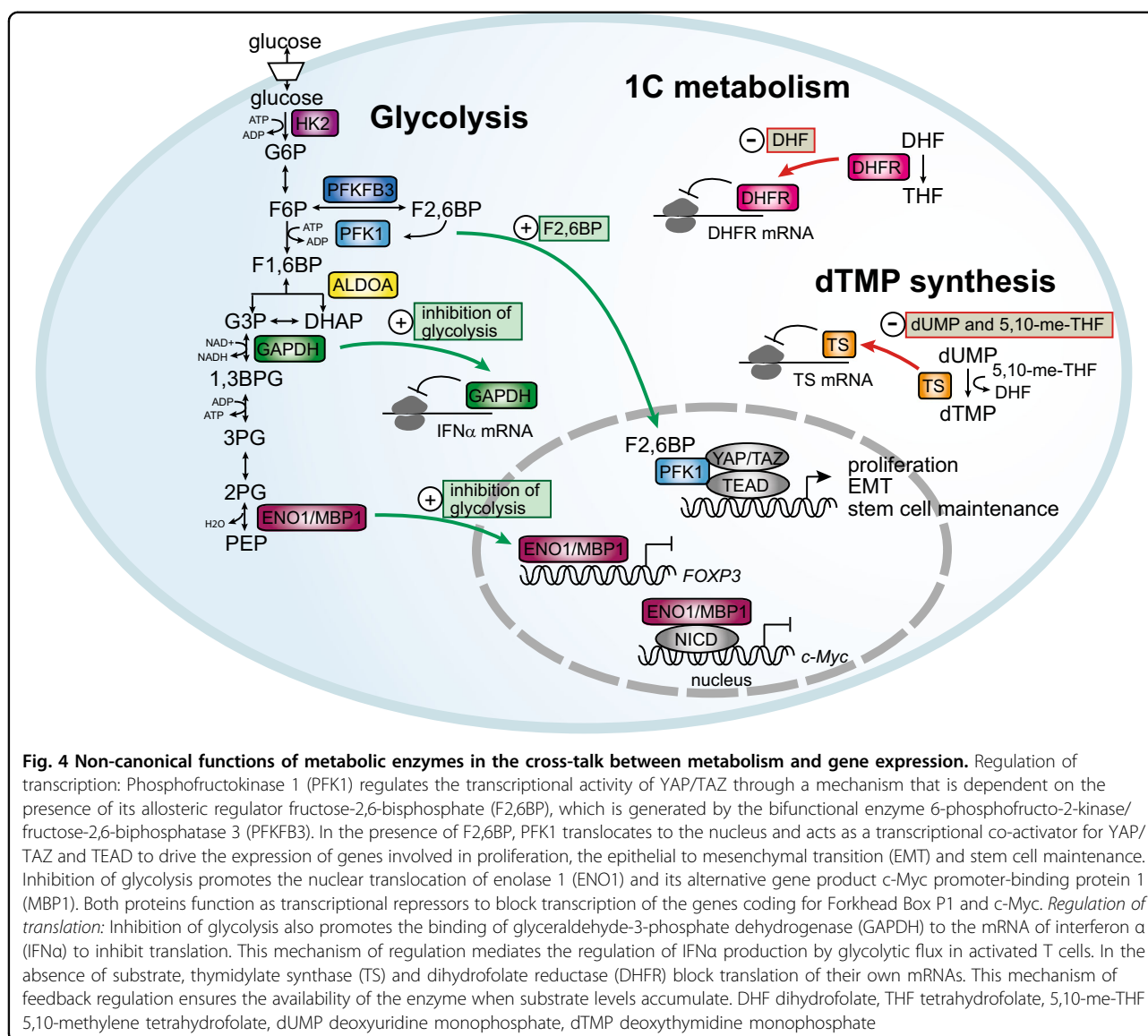
exclusive. It will be interesting to see whether this also applies to the ENO1/MBP1-mediated repression of c-MYC expression in cancer cells.

PKM2 may integrate mitogenic signaling with the metabolic state of the cell

As discussed in the “Oncogenic non-canonical functions of metabolic enzymes” section of this review, the non-canonical functions of PKM2 include protein kinase and transcription factor (in its dimeric form). The oligomeric state of PKM2 is allosterically regulated by F1,6BP and altering the amount of glucose available to the cell impacts both the intracellular F1,6BP levels and the oligomeric state of PKM2^{8,10}. Some of the non-canonical functions performed by dimeric PKM2 are preceded by posttranslational modifications that lock PKM2 into the dimeric state^{13,96}, effectively abolishing the metabolic regulation of the PKM2 oligomeric state and the non-canonical functions carried out by the PKM2 dimeric state. However, there are indications that some of the non-canonical functions of PKM2 in the nucleus can be regulated by the metabolic state of the cell. In a study investigating the nuclear interaction of PKM2 and Oct4 in glioma spheroids enriched in cancer stem cells (CSC), treatment with the pyruvate dehydrogenase kinase (PDK) inhibitor dichloroacetate (DCA) induced the nuclear translocation of PKM2 and promoted the interaction between PKM2 and Oct4²⁶. Likewise, the finding that PKM2 uses PEP as a phosphate donor for its protein kinase activity raises the possibility that the kinase function of PKM2 is dependent on glycolytic flux via the availability of PEP; PKM2 may, therefore, integrate mitogenic signaling with metabolic activity. In this scenario, PKM2 protein kinase activity would not only represent a driver for proliferation but would also act as an indicator for a nutrient- and energy-rich metabolic state that can support macromolecule biosynthesis and cell proliferation. PKM2 would thus act as a safety valve that only propagates mitogenic signals when nutrient levels are sufficient to support proliferation.

Metabolic enzymes as RNA binding proteins

Through recent mRNA interactome studies in HeLa, HEK, and mESC cells, it is evident that multiple enzymes from intermediary metabolism also function as mRNA-binding proteins (reviewed in ref.⁹⁷). The functional importance of most of these interactions is not fully understood, as the identity of many of the interacting mRNAs remains largely unknown. However, the non-canonical functions as mRNA-binding proteins of some metabolic enzymes have been studied in greater detail (reviewed in refs.^{98,99}). In some enzymes, the catalytic activity and mRNA-binding activity are interdependent, providing a potential mechanism for the functional



linkage of metabolic activity and posttranscriptional regulation.

Of the metabolic enzymes that can also bind mRNA, the best characterized is GAPDH, which has been shown to interact with numerous, different mRNAs and regulates their stability or efficiency of translation (reviewed in ref.¹⁰⁰). For some of these interactions, the effect of the catalytic activity of GAPDH on its non-canonical function as an mRNA-binding protein has not been addressed. However, it has been shown that in monocytes and activated T cells, elevated glycolytic activity promotes cytokine production in a manner that is dependent on GAPDH mRNA binding^{101,102}. In T cells, the switch from oxidative phosphorylation to aerobic glycolysis during T cell activation is required in order to produce the cytokines IFN- γ and IL-2. When activated T cells are cultured

on galactose instead of glucose, which results in reduced glycolytic activity, GAPDH binds to the IFN- γ and IL-2 mRNAs and suppresses their translation. However, when the cells are returned to glucose-containing media and can switch to aerobic glycolysis, GAPDH binding to IFN- γ and IL-2 mRNA is reduced and translation occurs (Fig. 4)¹⁰². Furthermore, GAPDH-mediated suppression of cytokine production in T cells cultured in galactose was reversed by supplementing the medium with G3P, the substrate for GAPDH in glycolysis, showing that cytokine production is controlled by the competition between glycolytic flux and GAPDH mRNA binding¹⁰².

Thymidylate synthase (TS) and dihydrofolate reductase (DHFR) are enzymes involved in the de novo synthesis of dTMP and one-carbon metabolism, respectively. Both enzymes have been found to bind their own mRNAs and

repress translation (Fig. 4)¹⁰³. In both cases, mRNA binding is inhibited by the respective substrate of the enzyme: dUMP and 5,10-methylenetetrahydrofolate for TS and dihydrofolate for DHFR. This type of auto-regulation couples the translation of TS and DHFR with the availability of their substrates, thereby facilitating enzyme production when the need for its enzymatic activity is highest, such as during DNA synthesis¹⁰³. Interestingly, TS has also been reported to bind other mRNAs including the p53 mRNA and overexpression of TS leads to reduced p53 translation¹⁰⁴.

Another example of mRNA-binding enzymes is mevalonate kinase (MVK), which catalyzes the conversion of mevalonate to phospho-mevalonate as part of the cholesterol biosynthesis pathway. MVK, also called LHR receptor mRNA binding protein (LRBP) binds to the luteinizing hormone receptor (LHR) mRNA, resulting in its degradation^{105,106}. LHR mRNA stability is regulated by both estrogen and ERK1/2 signaling, which increase and decrease MVK levels, respectively^{106,107}. The binding of MVK to LHR mRNA is inhibited by mevalonate, the substrate for MVK, while treatment with mevastatin, an inhibitor of mevalonate synthesis, results in increased LHR expression. Furthermore, the same amino acids in MVK that are essential for its catalytic function are also required for its mRNA-binding activity¹⁰⁸. These data indicate that mRNA-binding activity and the catalytic activity of MVK are mutually exclusive^{98,105}. Considering the frequent deregulation of enzymes of the mevalonate pathway in various human cancers¹⁰⁹, it will be interesting to see whether MVK might also interact with other mRNAs to modulate the expression of cancer-relevant genes.

Concluding remarks

It is now evident that the non-canonical functions of metabolic enzymes play important roles in cellular processes that are closely linked to oncogenic signaling and cell transformation. These additional activities need to be considered when studying metabolic alterations in cancer cells. One example is the large-scale screening studies that aim to identify metabolic enzymes that are essential for the survival of cancer cells. Such screens generally use gene silencing or knockout strategies and it is likely that, in some cases, the essentiality of the identified enzymes is caused by their non-canonical activities. In such a case, drug screens are faced with the additional challenge that non-canonical functions often depend on protein/protein interactions, which are difficult to target with small molecules. One possible solution for this problem could be the use of drugs that induce the selective ubiquitylation and degradation of a target protein through molecules known as proteolysis targeting chimeras (PROTACs)¹¹⁰. Indeed, PROTAC strategies should be particularly successful for the targeting of metabolic enzymes, as these

proteins contain catalytic centers that can selectively bind small molecule ligands. Another strategy for the targeting of non-canonical functions of metabolic enzymes could be to exploit the known mechanisms of allosteric regulation. For example, compounds that lock PKM2 into its highly active tetrameric conformation have already been shown to block tumor growth in a xenograft model of non-small cell lung cancer¹¹¹. In addition to inducing constant activation of its catalytic activity, these compounds should also block the non-canonical functions of PKM2 that may depend on the formation of the dimer. Non-canonical enzyme functions that are regulated by substrate availability (as in the case of mRNA binding by TS or DHFR) or that require the binding of a specific ligand (as in the case of transcriptional coactivation by PFK1) can be targeted by small molecules that either block or mimic these interactions. Finally, non-canonical enzyme functions that are regulated by metabolic flux or respond to cellular signaling pathways can be modulated by anti-metabolites or targeted therapies. However, in most cases, it will be challenging to confirm that the therapeutic effect is indeed linked to the non-canonical function of the enzyme. Additional research is needed to reveal the full extent of the contribution of non-canonical functions of metabolic enzymes in cancer and to identify suitable strategies for their therapeutic targeting.

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Conflict of interest

The authors declare that they have no conflict of interest.

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