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Mini-Review

The Warburg-like regulation of PGAM in tumor

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ABSTRACT

One of the distinctive metabolic features in cancerous cells is the tendency to consume high amounts of glucose in lactate production. The enhancement of glycolysis is clinically established and is known as the Warburg effect, while its post-transcriptional regulation is not clear. The conversion of 3-phosphoglycerate to 2-phospho-glycerate in the glycolytic pathway is catalyzed by phosphoglycerate mutase (PGAM), which is upregulated in many human cancer cells. In this review, we focus on the recent observations in the regulation of PGAM. We recently identified Mdm2 as a ubiquitin ligase for PGAM. Serine 118 residue in PGAM is phosphorylated by Pak1 kinase under senescence-inducing stress, which triggers the ubiquitination of PGAM. Oncogenic stimuli such as Ras-G12V provoke premature senescence, accompanied by the proteolytic degradation of PGAM by wild type Mdm2, while the oncogenic combination such as Ras-G12V+Mdm2-M459I transforms primary cells by stabilization of PGAM. Thus, Mdm2 functions as a tumor suppressor to attenuate the Warburg effect.

KEYWORDS: Warburg effect, senescence, glycolysis, PGAM, Pak1, Mdm2, phosphorylation, ubiquitination

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INTRODUCTION

In normal cells, glycolysis is an essential metabolic process for energy production. Extracellular glucose is taken into the cytoplasm via glucose transporters (Figure 1). Pyruvate, converted from glucose, would enter the tricarboxylic acid cycle (TCA cycle) to produce 36 molecules of adenosine triphosphate (ATP) by the mitochondrial oxidative phosphorylation. Alternatively, when oxygen is limited, glucose is metabolized through anaerobic condition, which generates lactate with production of two ATPs from one molecule of glucose. Dr. Otto Heinrich Warburg, proposed the hypothesis referred to as the Warburg effect which is the observation that cancer prefers the anaerobic glycolysis rather than aerobic one [1]. In the early days, it was presumed that enhanced glycolysis could be coupled with the impairment in mitochondrial function [2]. But further studies by several groups have shown that mitochondrial function is rather intact in most of cancer cells, which brings into argument whether mitochondrial dysfunction has the causal effect on the Warburg effect.

It has been assumed that cancerous cells upregulate glycolytic metabolism partly to adjust to the hypoxic conditions *in vivo*, as hypoxia-inducible transcription factor (HIF-1), a transcriptional regulator for most of glycolytic enzymes [3], is activated under hypoxic conditions. However, the

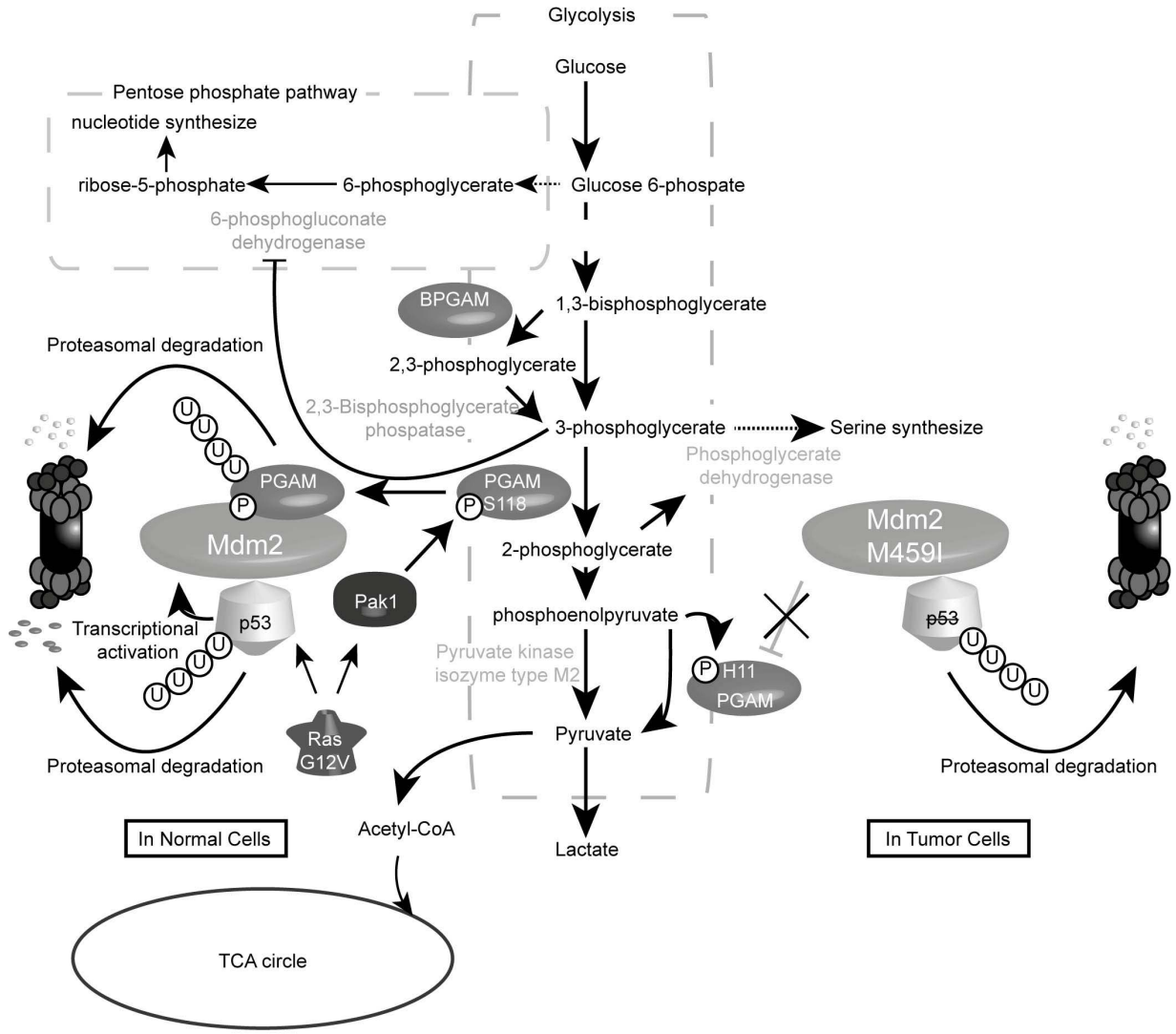

Figure 1. Ubiquitination of PGAM by Mdm2 as a tumor suppressor. The details are described in the text.

Warburg effect could not be explained simply by cellular adjustment to hypoxia, because cancerous cells restore high glycolytic flux even in the standard culture condition with 20% oxygen [4]. Indeed, cancer cells prefer glycolysis both for energy production and for biomass synthesis [5], as glucose-6-phospate, an intermediate metabolite in glycolysis, enters into the pentose phosphate pathway (PPP) to produce NADPH and ribose-5-phosphate for nucleotide synthesis (Figure 1).

In an earlier study we showed the intriguing relationship between glycolytic metabolism and cellular senescence [6]. Most of the primary somatic cells, except for stem cells, have limited replicative capacity under normal culture conditions and cause irreversible cell cycle arrest called replicative senescence, after their proliferative exhaustion [7]. Moreover, premature senescence is caused by induction of oncogenic stress [8], DNA damage [9], oxidative stress [10], exposure to secreted cytokines [11-13] etc. Interestingly, the reduction of glycolytic flux was observed in the senescent cells both in mouse and human [14, 6], while an inhibition of glycolytic flux also induced premature senescence [6].

Among others, the glycolytic enzyme phosphoglycerate mutase (PGAM), converting 3-phosphoglycerate into 2-phosphoglycerate (Figure 1), is a

key enzyme to link the Warburg effect to cancerous immortalization, as its ectopic expression immortalizes primary mouse embryonic fibroblasts (MEFs) [6]. While the most of the glycolytic enzymes are transcriptionally regulated by HIF-1α [3] or c-Myc [15], PGAM is an outlier, which is not subject to regulation by them [3, 16]. Enzymatic activity of PGAM is upregulated in many cancerous tissues, including the lung, colon, liver, and breast cancer cells [17-20]. In addition, the ablation of PGAM activity in cancerous cells induces cell death, implicating that PGAM might be an attractive target for therapeutic intervention against tumorigenesis [21-23] (Figure 2).

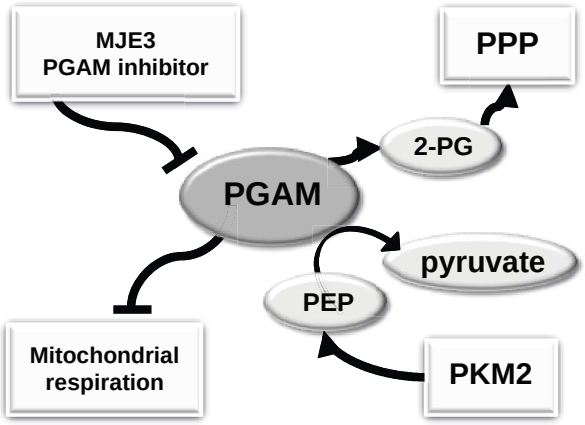

Figure 2. Physiological impact of PGAM relevant to cancer metabolism. The details are described in the text.

The metabolic modulation of glycolysis via PGAM

PGAM belongs to the phosphoglycerate mutase family in the glycolytic metabolism. There are two major groups in this enzyme, bisphosphoglycerate mutase (BPGAM) and monophosphoglycerate mutase (MPGAM). BPGAM catalyzes the formation of 2,3-bisphosphoglycerate, which is an intermediate product in the generation of 3-phosphoglycerate from 1,3-bisphosphoglycerate in the glycolytic pathway (Figure 1). MPGAM catalyzes the conversion of 3-phosphoglycerate into 2-phosphoglycerate by transfer of a phosphate group (Figure 1). MPGAM consists of two classes; dPGAM and iPGAM, whose catalytic activity is dependent and independent on 2,3-bisphosphoglycerate, respectively. Mammalian tissues express only BPGAM and dPGAM [24, 25]. dPGAM functions as a dimer of PGAM proteins. Two isoforms of PGAM called brain-form PGAM (PGAM1) and muscle-form PGAM (PGAM2), were initially identified in the brain and muscle, respectively. Recent work on mRNA profiles in mice suggests that PGAM1 is abundant in brain, blood vessel, white adipose tissue and liver, while PGAM2 in muscle, skin, bone and lung [26]. PGAM1 has about 80% homology with PGAM2. Five amino acids His11, Glu19, Tyr26, Arg62, and His186 constitute an enzymatic active site in PGAM1, while one amino acid of the catalytic active site at position 26 in PGAM2 is Phe instead of Tyr in PGAM1. Both PGAM1 and PGAM2 display much similar enzymatic activity in tissue-cultured cells [6, 26].

Recent studies suggest that glycolytic metabolites affect PGAM activity. Pyruvate kinase catalyzes the conversion of phosphoenolpyruvate (PEP) into pyruvate (Figure 1). While M1 isoform of pyruvate kinase (PKM1) is ubiquitously expressed, pyruvate kinase isoenzyme type M2 (PKM2) is highly expressed in many cancers [27, 28], but its significance is rather controversial [29-31]. Vander Heiden *et al.* indicated an alternative glycolytic pathway to catalyze PEP into pyruvate in PKM2 expressing cells [32]. They observed that PEP is accumulated much more in these PKM2 expressing cells than in the PKM1 expressing cells, as the enzymatic activity of PKM2 is about half of PKM1. Accumulated PEP induces the histidine phosphorylation of PGAM in the His11 residue (Figure 1). The phosphorylated PGAM directly mediates conversion of PEP into pyruvate [32] (Figure 2). In this context, PGAM might work as a key element for higher glycolytic flux on proliferative PKM2-expressing cells.

Moreover, 3-phosphoglycerate and 2-phosphoglycerate, glycolytic metabolites, can also affect PPP. PGAM catalyzes the conversion of 3-phosphoglycerate into 2-phosphoglycerate [16]. Hitosugi *et al.* [33] demonstrated that PGAM knockdown in lung carcinoma cell line H1299 resulted in a significant reduction of glycolytic metabolism, followed by the decrease of PPP flux and cell proliferation. Once PGAM is inhibited, elevated 3-phosphoglycerate interacts with 6-phosphogluconate dehydrogenase that catalyzes the conversion of 6-phosphoglycerate into ribose-5-phosphate (Figure 1). Thus 3-phosphoglycerate

inhibits the binding of 6-phospholuconate dehydrogenase with its substrate, 6-phosphoglycerate (Figure 1). As a result, the PPP flux and the nucleotide synthesis are downregulated. In addition, reduction in 2-phosphoglycerate generation also inhibits the activity of phosphoglycerate dehydrogenase and inhibits serine generation from 3-phosphoglycerate (Figure 1).

Post-translational regulation of PGAM

Although the significance of PGAM in the Warburg effect is now well established, its regulation is not clear. While the most glycolytic enzymes are transcriptionally regulated by HIF-1α [3] or c-Myc [15], PGAM mRNA is less affected by HIF-1α or c-Myc, but its enzymatic activity is post-translationally regulated [21]. Pak1 kinase, whose gene is amplified in human tumors, phosphorylates PGAM (Figure 1). Pak1 is activated by Cdc42/ Rac1, members of the Rho family of GTPase, which is involved in many cellular processes, cell motility, actin re-organization, gene transcription, and apoptosis [34]. Recently, we showed that Pak1-mediated phosphorylation of PGAM promotes its ubiquitination and subsequent degradation via the proteasomal pathway (Figure 1) [26].

PGAM is post-transcriptionally regulated through phosphorylation-dependent ubiquitination by senescence-inducing stress, DNA damage or oncogenic stress [26]. Rapid degradation of PGAM in senescent cells causes dramatic reduction of glycolytic flux. Activated Pak1 during senescence induces the phosphorylation of PGAM on Ser118, and this phosphorylation promotes the interaction between PGAM and E3 ubiquitin ligase, Mdm2. PGAM is ubiquitinated by Mdm2, followed by its degradation via the proteasomal pathway (Figure 1; In Normal Cells). Mdm2 is well known as an oncogene for the following reasons. It was identified as a ubiquitin ligase for p53 tumor suppressor [35]. *Mdm2* gene has been observed to be amplified in several human cancer cells [36, 37]. In addition, Mdm2 is also reported as a ubiquitin ligase that induces the degradation of an accessary protein encoded in HIV-1 genome, Viral Infectivity Factor (Vif) via the ubiquitin-proteasomal pathway [38], which causes p53-dependent cell cycle arrest in host cells [39]. The activation of p53 during senescence induced by DNA damage or direct oncogenic signaling, would negatively regulate glycolytic flux via the promotion of Mdm2 mediated ubiquitination and degradation of PGAM.

It is worth noting that Mdm2 is also known as a tumor suppressor in primary cells. Ectopic expression of Mdm2 in primary cells is reported to induce cell cycle arrest [40], and apoptosis under stress such as DNA damage [26]. We searched oncogenic mutations of Mdm2 in COSMIC database (the Catalogue of Somatic Mutations in Cancer, http://cancer.sanger.ac.uk/ cancergenome/projects/cosmic/) to verify its tumor suppressive function. Mdm2-Y281H and -W329G, mutations in inhibitory domain, partly lose its ubiquitin ligase activity against PGAM but not against p53. The RING finger domain mutation, Mdm2-M459I completely abolished its ligase activity both against PGAM and p53 (Figure 1), even though the M459I mutant still sustains the ability to inhibit p53 transactivation.

It is worth noting that, oncogenic Ras-G12V provokes premature senescence in primary cells [8], accompanied by downregulated PGAM activity (Figure 1), while it co-operates with Mdm2-M459I and PGAM for cancerous transformation. Thus Mdm2 attenuates the Warburg effect as a tumor suppressor in cells exposed to a stress of oncogene-induced senescence.

There are several reports that indicate the importance of PGAM as a novel therapeutic target for cancer treatment [20, 32, 33, 41]. The regulatory mechanism of PGAM by Mdm2 might provide new insights for therapeutic interference against tumorigenesis.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest with regard to this paper.

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