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The Pentose Phosphate Pathway as a Potential Target for Cancer Therapy

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Abstract

During cancer progression, cancer cells are repeatedly exposed to metabolic stress conditions in a resource-limited environment which they must escape. Increasing evidence indicates the importance of nicotinamide adenine dinucleotide phosphate (NADPH) homeostasis in the survival of cancer cells under metabolic stress conditions, such as metabolic resource limitation and therapeutic intervention. NADPH is essential for scavenging of reactive oxygen species (ROS) mainly derived from oxidative phosphorylation required for ATP generation. Thus, metabolic reprogramming of NADPH homeostasis is an important step in cancer progression as well as in combinational therapeutic approaches. In mammalian, the pentose phosphate pathway (PPP) and one-carbon metabolism are major sources of NADPH production. In this review, we focus on the importance of glucose flux control towards PPP regulated by oncogenic pathways and the potential therein for metabolic targeting as a cancer therapy. We also summarize the role of Snail (*Snai1*), an important regulator of the epithelial mesenchymal transition (EMT), in controlling glucose flux towards PPP and thus potentiating cancer cell survival under oxidative and metabolic stress.

Key Words: Pentose phosphate pathway, NADPH, Glucose-6-phosphate dehydrogenase, Snail, Epithelial-mesenchymal transition

INTRODUCTION

For more than half a century, cancer metabolism studies have focused on cell proliferation through anabolic pathways. Warburg described the major role of aerobic glycolysis in proliferating cancer cells converting glucose to lactate, generating biomass such as amino acids, fatty acid, and nucleotides (Warburg, 1956; Vander Heiden *et al*., 2010). Unlike *in vitro*, cancer cells *in vivo* are repeatedly subjected to resource-limited microenvironments of nutrition depletion, hypoxia and acidosis which induce in the cells a more quiescent, catabolic slow-life (slow proliferating cancer cells) stage which enables survival under metabolic stress (Ward and Thompson, 2012; Aktipis *et al*., 2013). Because the metabolic stress inevitably induces ROS-mediated oxidative stress, even under hypoxic condition (Chandel *et al*., 1997, Kang *et al*., 2015) nicotinamide adenine dinucleotide phosphate (NADPH) homeostasis is critical for cancer cell survival under starved microenvironment. It should be noted that current standard cancer therapeutics, such as chemotherapy and radiotherapy, also significantly induces oxidative stress in cancer cells, and antioxidant capacity provides therapeutic resistance in cancer cells (Ramanathan *et al*., 2005; Diehn *et al*., 2009; Decrock *et al*., 2017). While little is known about the metabolic pathways promoting NADPH homeostasis in cancer, emerging evidence reveals an important molecular network regulating metabolic flux into the pentose phosphate pathway for NADPH generation.

OVERVIEW: PENTOSE PHOSPHATE PATHWAY (PPP) AND GLYCOLYSIS

Non-dividing normal differentiated cells mainly depend on mitochondrial oxidative phosphorylation to generate ATP (adenosine 5'-triphosphate). However, most human cancer cells metabolize the glucose through glycolysis (known as Warburg effect, for review glycolysis, see Vander Heiden *et al*., 2009), producing pyruvate and lactate as final metabolites in cells (Fig. 1). Although the glycolysis is inefficient in produc-

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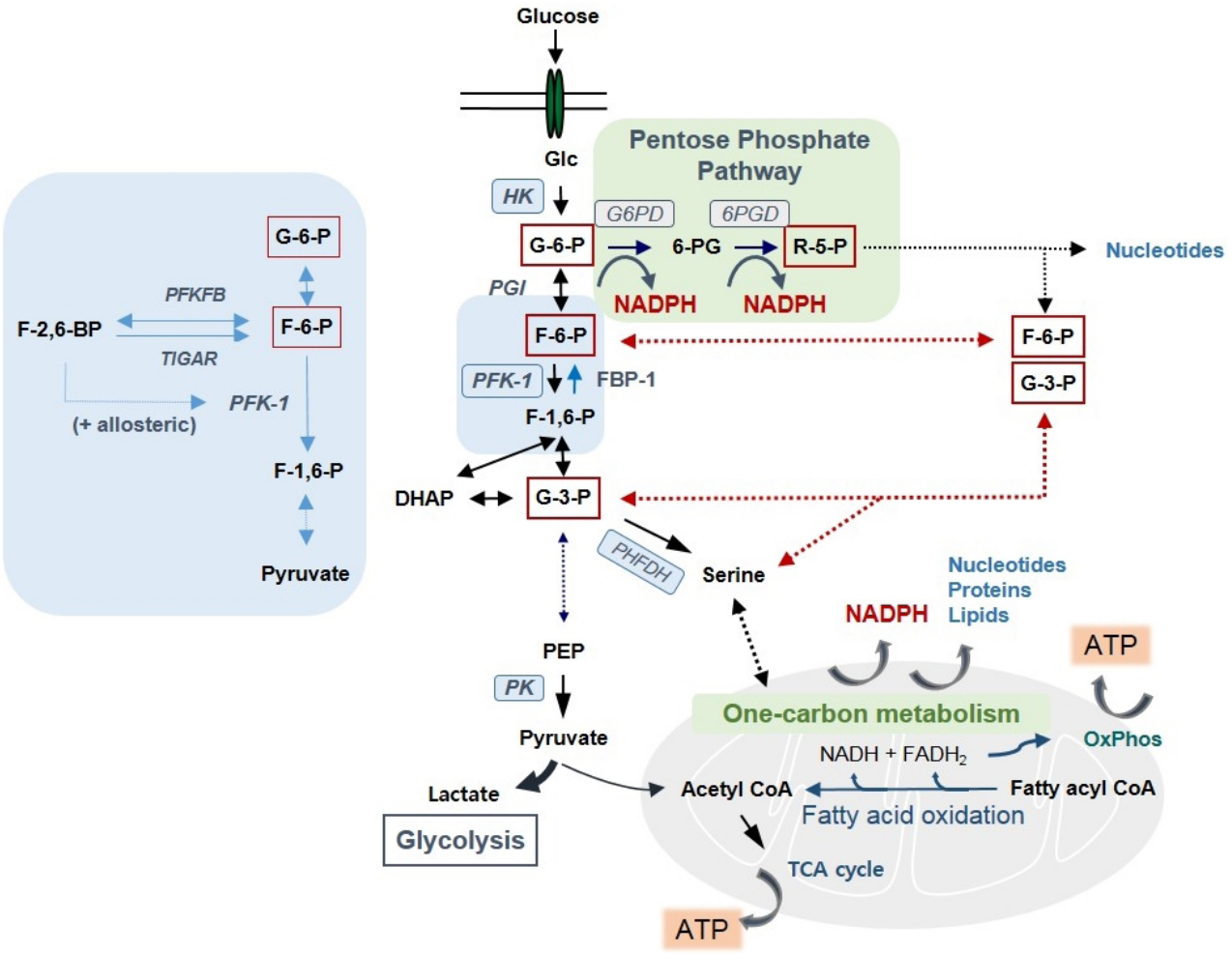


Fig. 1. The glycolysis and pentose phosphate pathways (PPP) are tightly connected. The glucose entering the in cell membrane is rapidly phosphorylated by HK and converted to G-6-P. The G-6-P is metabolized either by the glycolytic pathway, generating pyruvate and lactate, or by PPP to produce NADPH. The F-6-P and G-3-P generated from the non-oxidative branch of PPP can enter glycolysis or gluconeogenesis. The NADPH is generated from oxidative PPP in cytosolic space and serine-driven one carbon metabolism in mitochondria. Arrows indicate irreversible enzymatic steps, and bi-directional arrows indicate interconverting reversible reactions determined by substrate concentration. Inset (left) shows the feed-forward regulatory loop of PFK-1 as a rate-limiting step of glycolytic flux. G-6-P: glucose-6-phosphate, F-6-P: fructose-6-phosphate, F-1,6-P: fructose1,6-bisphosphate, F-2,6-BP: fructose2,6-bisphosphate, G-3-P: glyceraldehyde-3-phosphate, PEP: phosphoenolpyruvate, 6-PG: 6-phosphoglucolactone, R-5-P: Ribose-5-phosphate, *HK*: Hexokinase, *PGI*: phosphoglucose isomerase, *PFK-1*: phophofructokinase-1, *FBP-1*: fructose-1,6-bisphosphatase, *PK*: pyruvate kinase, *G6PD*: glucose-6-phosphate dehydrogenase, 6-PGD: 6-phosphogluconate dehydrogenase, *PFKFB*: 6-phosphofructo-2-kiase/fructose-2,6-biphosphatase, TIGAR: TP53-induced glycolysis and apoptosis regulator.

ing ATP, proliferating cancer cells under nourished condition utilize glucose in biomass production (such as amino acids, nucleotides, and fatty acids) to support new cells. Because the biologic aspects of glycolysis in proliferating fast-life of cancer cells are well-known, we mainly focused on the importance and regulatory network of PPP in supporting slow-life of cancer cells.

The PPP (also known as the phosphogluconate pathway or the hexose monophosphate shunt) branched form glycolysis at the first committed step of glucose-6-phosphate (G-6-P) (Fig. 1). The PPP promotes the oxidative decarboxylation of G-6-P (oxidative PPP branch), yielding ribulose-5-phosphate (Ru-5-P). The Ru-5-P is isomerized into ribose-5-phosphate (R-5-P), which either serves as a nucleotide precursor or is metabolized through the non-oxidative PPP branch to produce fructose-6-phosphate (F-6-P) and glyceraldehyde-3-phosphate (G-3-P) via multiple enzymatic steps (for a detailed review of PPP, see Riganti *et al*., 2012 and Patra and Hay, 2014). Because F-6-P and G-3-P are also provided by the glycolytic process (Fig. 1), the NADPH from the oxidative phase is a unique biomolecule from PPP flux compared to glycolysis. The NADPH is essential for scavenging the reactive oxygen species (ROS) mainly derived from oxidative phosphorylation. In contrast, proliferating cells under nourished condition also have a high need for PPP activity to provide ribose-5-phosphate generating nucleotides and NADPH. The cancer cells in fast-life mainly consume NADPH during fatty acid synthesis (FAS) (Riganti *et al*., 2012). Thus, the regulatory network of PPP flux constitutes an important metabolic adaptation in various environmental contexts in human cancer.

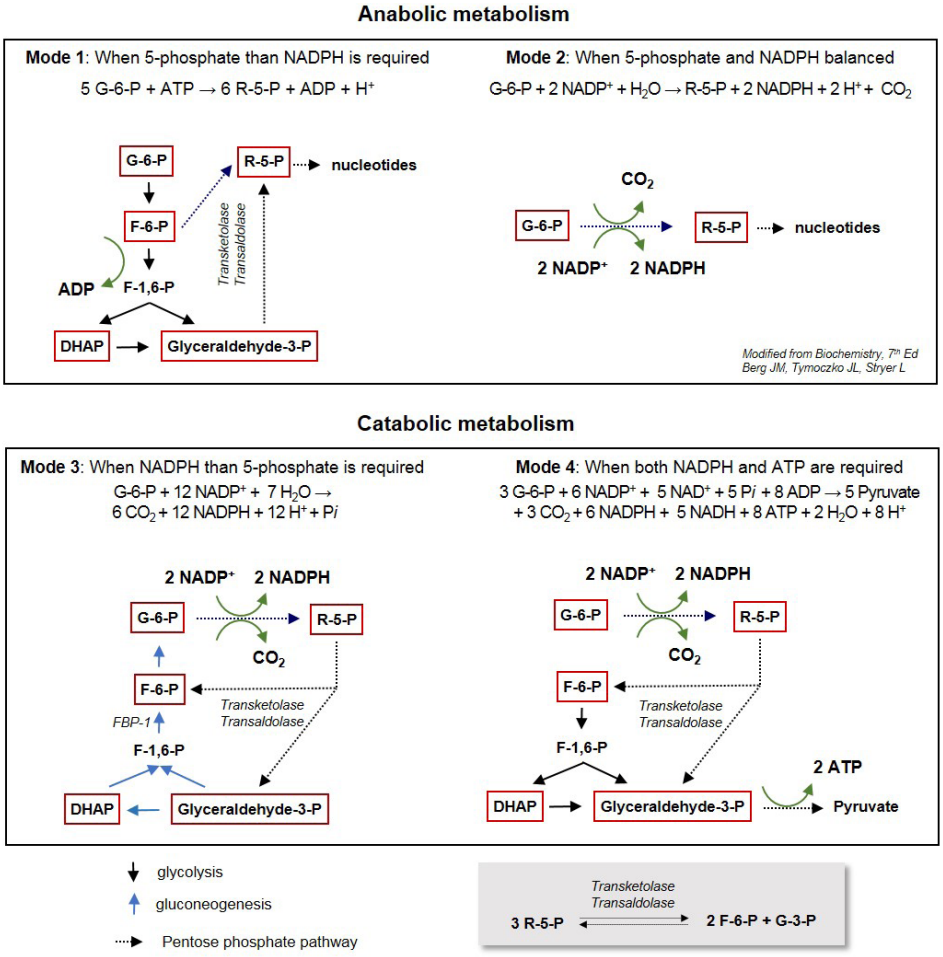


Fig. 2. Diverse flux modes interconnecting glycolysis and PPP in different metabolic situations (modified from Berg *et al.*, 2010). Major metabolites are shown in red box and the stoichiometry of each mode is denoted. The PPP and glycolysis are reversibly linked by bi-directional transketolase (TKT) and transaldolase (TALDO1), as shown below.

PPP IS TIGHTLY CONNECTED TO GLYCOLYSIS

The glucose metabolism consists of highly interconnected pathways, and the key junctions between glycolysis and PPP being three important biomolecules: G-6-P, pyruvate, and acetyl CoA. Upon entering cells, glucose is rapidly phosphorylated to G-6-P by hexokinase (HK), and is subsequently metabolized toward glycolysis or the PPP. Although the PPP and glycolysis are divided from G-6-P, the metabolic circuit of both pathways is tightly connected, and a different flux mode is active depending on metabolic demands. The reversible nature of enzymes through allosteric regulation in the non-oxidative branch enables the PPP to engage glycolysis to adapt to the metabolic demands of cells (Fig. 1). The R-5-P, a product of oxidative PPP, is converted into G-3-P and F-6-P by *transketolase* (TKT) and *transaldolase* (TALDO1), creating a reversible metabolic link between PPP and glycolysis (Patra and Hay, 2014). Diverse metabolic circuits between PPP and glycolysis depend on the fundamental metabolic need for NADPH, R-5-P, and ATP (Berg *et al.*, 2010) (Fig. 2). When rapidly proliferating cells, such as cancer cells under nourished environment, require R-5-P rather than NADPH or ATP for the synthesis of nucleotide precursors supporting a fast-life (Mode I), F-6-P and G-3-P produced from glycolysis can enter the PPP via TKT and TALDO1. In this process, 5 moles of G-6-P are converted to 6 moles of R-5-P by consuming ATP. When the G-6-P enters the PPP directly, two moles of NADPH and one of R-5-P are generated from 1 mole of G-6-P in oxidative PPP (Mode II). When the cell needs a high level of NADPH rather than R-5-P, the NADPH is efficiently produced by interplay between PPP and glycolysis (Mode III). In this flux mode, the first step is the same as Mode II, wherein 6 moles of G-6-P can generate 12 moles of NADPH and 6 moles of R-5-P. These 6 moles of R-5-P are readily converted into 4 moles of F-6-P and 2 moles of G-3-P by TKT and TALDO1, and 5 moles of G-6-P are synthesized from both F-6-P and G-3-P via the gluconeogenic pathway. This flux, also known as recycling PPP, can completely oxidize G-6-P to CO_2 with concomitant generation of NADPH. Although the flux amount can hardly be quantified in cells, the recycling PPP mode might be very important in cells requiring a high NADPH level, such as liver, fat tissue and neuron (Bouzier-Sore and Bolanos, 2015). This recycling flux mode should utilize gluconeogenesis, a reverse reaction of glycolysis, and FBP1 which converts F-1,6-BP to F-6-P as a rate-limiting enzyme in this flux. Note that PFK-1 and FBP1 interconvert F-6-P and F-1,6-P, representing rate-limiting bot-

tlenecks of glycolysis and gluconeogenesis, respectively (discussed below in detail). Lastly, F-6-P and G-3-P derived from PPP can enter the glycolytic pathway to pyruvate (Mode IV). In this flux mode, NADPH and ATP are concomitantly generated, and the pyruvate can be oxidized to further generate ATP in mitochondria. Collectively, the PPP and glycolysis are tightly linked by bidirectional reactions of TKT and TALDO1, and the cancer cells utilize diverse flux modes depending on metabolic demand and the environmental situation.

METASTATIC INEFFICIENCY DURING CANCER PROGRESSION

In nourished microenvironments, cancer cell proliferation is mediated via well-known aerobic glycolysis with an additional mitochondrial oxidative phosphorylation (Ox Phos) (Koppenol *et al*., 2011; Ward and Thompson, 2012). In this nourished condition, the cancer cell utilizes Mode I and Mode II of PPP flux, mainly to support anabolic metabolism.

Distant metastasis comprises the most common cause of cancer mortality. It may therefore seem illogical to consider metastasis as inefficient. Nevertheless, clinical and experimental evidence suggests that the metastatic progression of cancer cells is indeed highly inefficient (Tarin *et al*., 1984; Luzzi *et al*., 1998; Chambers *et al*., 2002; Valastyan and Weinberg, 2011). Unlike hematopoietic cells, cancer cells arising from epithelial tissue (so-called carcinoma) should attach to the extracellular matrix, and matrix detachment leads to ATP deficiency and metabolic stress due to loss of glucose transport (Wong *et al*., 2001; Schafer *et al*., 2009). Conversely, matrix-detached and extravasated carcinoma cells must survive for successful metastatic progression. To do so, the cancer cells essentially require ATP, mainly derived from Ox Phos, NADH and NADPH, as a reducing power against oxidative stress (Cha *et al*., 2015). While oxidative phosphorylation mainly compensates ATP depletion in starved cells, it concurrently generates intracellular ROS. Intracellular ROS are mainly produced from mitochondrial activity in complex I (NADH dehydrogenase) and complex III (ubiquinone-cytochrome c reductase) of the electron transporter chain (Finkel and Holbrook, 2000). Under resource-limited conditions, increased mitochondrial Ox Phos and increased levels of intracellular ROS inhibit fatty acid oxidation (FAO) and ATP generation in a negative feedback loop (Riganti *et al*, 2012; Nogueira and Hay, 2013), impeding cell survival. A constant supply of reductive equivalents, specifically NADPH, for ROS detoxification is the key to maintaining metabolic homeostasis under stressful circumstances. Thus, cells under metabolic stressed condition, such as metastatic cancer cells, mainly use Modes III and IV flux of PPP to support catabolic metabolism.

THE IMPORTANCE OF NADPH GENERATION IN CANCER CELLS

NADPH is the major electron donor in the reductive metabolic process. Although NADPH can be generated through several mechanisms, recent quantitative flux analysis indicates that NADPH is mainly provided by cytosolic oxidative PPP and mitochondrial serine-driven one-carbon metabolism entering in folate-methionine cycles (Locasale, 2013; Fan *et al*., 2014) (Fig. 1). The serine is biosynthesized from G-3-P dervied either from PPP and glycolysis, and is catalyzed by 3-phosphoglycerate dehydrogenase (PHGDH). Because the serine provides NADPH as well as amino acids, the importance of serine biosynthesis has recently emerged. Indeed, a small molecule inhibitor of PHGDH suppressed serine biosynthesis and *in vivo* tumor growth in PHGDH-dependent cells (Mullarky *et al*., 2016; Pacold *et al*., 2016). While the serine derived from G-3-P generate 30-40% of NADPH under nourished environment, it should be noted that FAS and DNA synthesis in proliferating cells consume most of the NADPH (Fan *et al*., 2014). In starved condition with shutdown of glucose uptake, the role of serine-driven NADPH biosynthesis and ATP generation is still unclear and further study is required in terms of cell survival during cancer progression. In this review, we mainly focus on flux control towards PPP by environmental cues and ongogenic mutation in cancer cells.

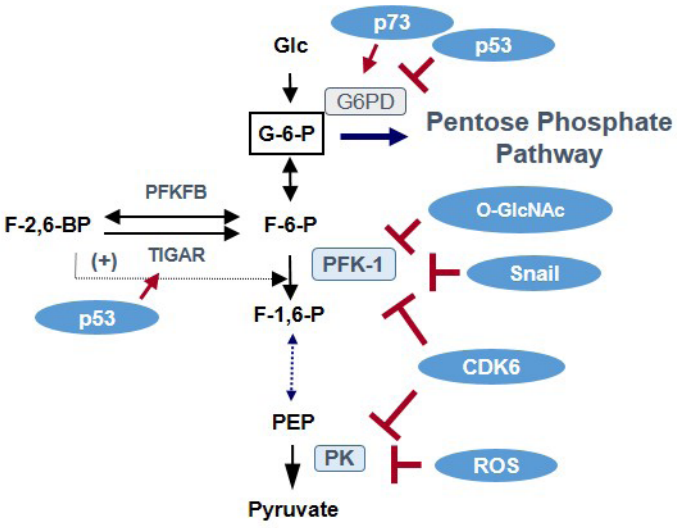
GLUCOSE FLUX CONTROL BETWEEN THE PPP AND GLYCOLYSIS IN CANCER

The glucose entering the PPP may vary from 5-30%, depending on tissue (Riganti *et al*., 2012). With enough glucose in non-dividing cells under normal physiologic condition, NADPH is mostly consumed during FAS. Therefore, high PPP flux is reached in liver, adipose tissue, lactating mammary glands, adrenal glands, and red blood cells (Cabezas *et al*., 1999; Riganti *et al*., 2012). The basal rate of the PPP, a major source of NADPH, mainly depends on the $NADP^+$/NADPH ratio; moreover, the G6PD enzyme is highly specific for $NADP^+$. Recently, metabolic regulation of PPP flux in human disease has gained significant attention due to the metabolic pathway's function against oxidative stress.

As noted earlier, matrix detachment of solid tumor cells induces ATP deficiency accompanied by increased ROS level due to the loss of glucose uptake. In this metabolic stress condition, the ERBB2 oncogene rescues the ATP depletion depending on glucose flux into PPP (Schafer *et al*., 2009) although the mechanistic link between ERBB2 and PPP is still unclear. There are several regulatory mechanisms involved in the flux control between glycolysis and PPP, including reciprocal interactions. First, PPP flux can be controlled by the glycolytic pathway. When the glycolytic pathway is inhibited, glucose re-flux towards the PPP is increased. Second, phosphofructokinase-1 (PFK-1) and pyruvate kinase (PK) convert irreversible enzymatic steps in glycolytic reactions. These enzymatic steps serve as important control sites of glycolytic flux via reversible allosteric control, post-translational modification (such as phosphorylation and *O*-glycosylation), and transcriptional regulation (Fig. 3). Therefore, genetic alterations in cancer and metabolic cues controlling glucose flux between PPP and glycolysis involve enzymatic steps catalyzed by (1) G6PD as a gatekeeper of PPP flux, and (2) PFK-1 and PK as a rate-limiting step of glycolysis (Fig. 3).

PFK-1 AND PK CONTROL FLUX BETWEEN THE PPP AND GLYCOLYSIS IN CANCER

PFK-1 is the most important control site in mammalian glycolysis as a first rate-limiting step, the kinase activity being in-



PPP flux regulators (Regulatory level)	Targets	PPP flux	References
ROS (Post-translational)	PK	↑	Anastasiou et al, 2011
O-GlcNAc (Post-translational)	PFK-1	↑	Yi et al, 2012
p53 (transcriptional & post-translational)	TIGAR, G6PD	↑ or ↓	Bensaad et al, 2006 Jiang et al, 2011
p73 (transcriptional)	G6PD	↑	Du et al, 2013
Snail (transcriptional)	PFK-1	↑	Kim et al, 2017
CDK6 (Post-translational)	PK, PFK-1	↑	Wang et al, 2017

Fig. 3. Oncogenic regulation of glucose flux into PPP.

hibited by ATP level in a negative feedback loop. Compared to G6PD and PK, the regulatory mechanism of PFK-1 is complex due to the presence of fructose 2,6-bisphosphate (F-2,6-BP), which forms in a reaction catalyzed by PFK-2, which acts as a potent activator of PFK-1 by increasing the affinity of PFK-1 to F-6-P and decreasing the inhibitory effect of ATP (Fig. 3). PFK-2 is a bi-functional enzyme that catalyzes the synthesis of F-2,6-BP from F-6-P with the N-terminal kinase domain and also catalyzes the reverse hydrolysis reaction with the C-terminal phosphatase domain (for a review of PFK-2, see Ros and Schulze, 2013). There are several PFK-2 isoenzymes in mammals, PFKFB1 to PFKFB4. Increased levels of F-2,6-BP allow cancer cells to maintain a high glycolytic activity independent of ATP levels (Berg *et al*, 2010). Therefore, F-2,6-BP level can be controlled by pharmacologic inhibition of N-terminal kinase activity on PFKBPs. Indeed, a small molecule inhibitor of PFKFB3, 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO), suppressed glycolytic metabolites such as lactate, ATP, and NADH while also attenuating the proliferation of cancer cells (Clem *et al*., 2008); the effect of 3PO on PPP flux is not yet known. Interestingly, PFKFB4 was identified as a therapeutic target of cancer, especially in prostate cancer (Ros *et al*., 2012). Knockdown of PFKFB4 leads to a significant increase in F-2,6-BP in cancer cells followed by decreasing NADPH level and increasing ROS with cancer cell death. As a well-known tumor suppressor in human cancer, p53 is involved in PFK-1 activity via transcriptional upregulation of TIGAR (TP53-induced glycolysis and apoptosis regulator) (Bensaad *et al*., 2006). Because the TIGAR possesses only the bisphosphatase active site lacking N-terminal kinase domain of PFKFB, it degrades F-2,6-BP cellular levels and thus inhibits PFK-1 activity and glycolysis (Li and Jogl, 2009; Mor *et al*., 2011). The inhibition of glycolysis by TIGAR increases PPP flux and NADPH levels with enhanced ability to regulate cellular ROS level and oxidative stress (Bensaad *et al*., 2006; Cheung *et al*., 2012; Ko *et al*., 2016). While the role of TIGAR in PPP flux is clear, it should be noted that p53 is involved in various levels of metabolic PPP circuitry other than TIGAR (for the effect of p53 on metabolism, see Kruiswijk *et al*., 2015).

As a gate-keeper of glycolysis, the PFK-1 constitutes an important control step regulating glucose flux between PPP and glycolysis. PFK-1 in mammal is encoded by three genes:

PFKL and PFKM, expressed in liver and muscle respectively, and PFKP, mainly expressed in platelet, each encoding a different isoform. In human cancer, the PFKP isoform prevails over PFKM or PFKL and is a major controlling step of aerobic glycolysis in human cancer (Kim *et al*., 2017). Post-translational O-GlcNAcylation on Ser residue of PFK-1 in response to hypoxia inhibits its kinase activity and redirects glucose flux towards the PPP, conferring a selective advantage against oxidative stress on cancer cells (Yi *et al*., 2012). Recently, cyclin D3-CDK6 complex has been found to phosphorylate and inhibit the PFKP in cancer (Wang *et al*., 2017). This inhibition of PFKP by CDK6 also redirects glucose flux into the PPP, providing a pro-survival function under metabolic stress. Snail, a key regulator of cancer EMT, suppresses glycolysis by transcriptional repression of PFKP and regulates glucose flux toward PPP, allowing cancer cell survival under metabolic stress condition similarly to CDK6 (Kim *et al*., 2017). Knockdown of PFKP significantly suppressed glycolytic flux with G0/G1 cell cycle arrest and ceased cell proliferation. However, suppression of PFKP significantly increased cancer cell survival under metabolic starved condition by increasing PPP flux and NADPH generation. Cancer cells in which endogenous PFKP was dynamically knockdowned exhibited increases resistance to oxidative stress as well as metastatic potential *in vivo* (Kim *et al*., 2017). Thus, suppression of PFKP during EMT provides cell survival advantage via Mode III flux. Note that FBP1, which catalyzes the reverse reaction of PFKP and constitutes a gatekeeper of gluconeogenesis, is also downregulated in many types of human cancer (Dong *et al*., 2013; Li *et al*., 2014; Zhang *et al*., 2016). Interestingly, Snail or ZEB1 EMT inducer also suppresses FBP1 expression, and restoring FBP1 in cancer cells inhibits invasion and tumorigenic potential. Paradoxically, loss of FBP1 decreases cellular ROS level similarly to suppression of FPKP. Although whether and how FBP1 controls flux into PPP remains unclear, the loss of FBP1 in cancer allows high glycolytic flux under nourished environment and may provide survival advantage with Mode IV flux under starved condition by generating NADPH and ATP together. Therefore, as rate-limiting steps of glycolysis and gluconeogenesis, the bidirectional suppression of PFKP and FBP1 by Snail repressor may play important role in regulating flux control in response to metabolic demands of cancer cells.

PK, the enzyme catalyzing the last irreversible step in glycolysis, also controls the glucose flux of glycolysis. In mammals, several isoforms of PK are encoded by different genes: L type in liver and the M type in muscle and brain. In human cancer, the M2 isoform of PK (PKM2) is exclusively expressed similarly to PFKP, and the PKM2 is phosphorylated by tyrosine kinase, resulting in inhibition of PKM2 kinase activity (Christofk *et al*., 2008a, 2008b). Interestingly, intracellular ROS and CDK6 directly inhibit PKM2 activity via oxidation and phosphorylation, respectively (Anastasiou *et al*., 2011; Wang *et al*., 2017). The inhibition of PKM2 by ROS or CDK6 suppresses glycolytic flux together with increased PPP flux and NADPH generation, providing a survival advantage to cancer cells (Fig. 3).

G6PD IS A PPP FLUX CONTROLLER IN CANCER

The PPP comprises two phases: the oxidative and the nonoxidative branches. Glucose-6-phosphate dehydrogenase (G6PD) generates NADPH in the oxidative PPP by oxidizing G-6-P to 6-phosphogluconolacton (6-PG). A second NADPH and CO_2 are generated downstream via oxidative decarboxylation of 6-phosphogluconic acid by 6-phosphogluconic dehydrogenase (6PGD), the last step of the oxidative branch producing ribulose/ribose-5-phosphate (R-5-P) (Riganti *et al*., 2012; Stanton, 2012). The nonoxidatvie branch comprises a series of reversible reactions by the allosteric enzymes to adapt to the metabolic demands of cells. As noted earlier, F-6-P and G-3-P can pass in either direction between the nonoxidative PPP branch and the glycolytic pathway (Fig. 1). Therefore, G6PD is the first committed enzyme for NADPH generation in the oxidative PPP branch; we only focus on the PPP oxidative branch, which maintains the redox balance in cancer cells.

Not surprisingly, cancer cells exhibit higher expression of G6PD than normal tissue (Bokun *et al*., 1987; Wang, Yuan *et al*. 2012). Several oncogenic signals are associated with PPP flux regulation through G6PD. The cytoplasmic p53 tumor suppressor directly interacts with G6PD and inhibits its activity, resulting in a decreasing both PPP flux and NADPH generation (Jiang *et al*., 2011). This observation merits special attention in terms of the role of p53 on PPP flux because p53's downstream target TIGAR increased the PPP flux, as noted earlier (Bensaad *et al*., 2006; Li and Jogl, 2009; Mor *et al*., 2011). The p53 also suppresses Snail via post-transcriptional regulation of miRNA-34 (Kim *et al*., 2011). Therefore, the role of p53 tumor suppressor and its downstream targets in human cancer require further study in terms of metabolic flux into glycolysis and PPP. Another member of the p53 family, p73, increases PPP flux and NADPH production via transcriptional activation of G6PD (Du *et al*., 2013). An earlier study revealed that epidermal growth factor (EGF) or platelet-derived growth factor (PDGF) increase G6PD activity, while the mechanistic link between receptor tyrosine kinase and G6PD is not well understood (Stanton *et al*., 1991). These observations indicate that G6PD is a key controller of PPP flux regulated by many oncogenes, and further investigation is required into G6PD regulation by oncogenes providing proliferation and survival advantage of cancer cells.

G6PD AS A THERAPEUTIC TARGET IN CANCER CELLS

Emerging evidence suggests that G6PD, a gatekeeper of PPP, is a potential therapeutic target of human cancer. Prior to discussing it as a cancer target, we should note the extensive epidemiological background of G6PD deficiency, the most common human genetic alteration, which has been studied for the past 90 years.

The PPP was first described in the 1920s, when the introduction of new antimalarial drugs such as primaquine led to drug-induced hemolytic anemia (cited from Riganti *et al*., 2012). Subsequently, it was revealed that individuals with primaquine-sensitive hemolysis and lowered reduced-form of GSH were G6PD-deficient (Carson *et al*., 1956). In red blood cells lacking mitochondria as well as nucleus, the PPP is the exclusive route for NADPH production. Therefore, attenuated PPP due to G6PD deficiency renders red blood cells more vulnerable to drug-induced oxidative stress. G6PD-encoding genes map at the telomeric region of the distal long arm of

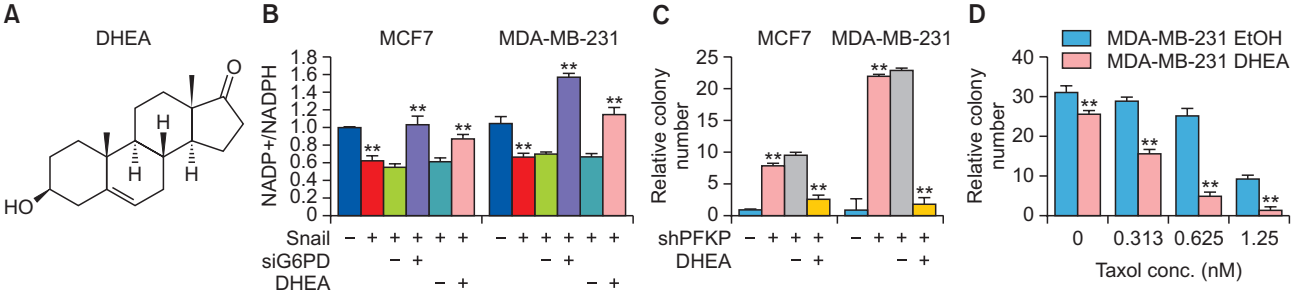


Fig. 4. The DHEA, an uncompetitive inhibitor of G6PD, regulated NADPH level and cancer cell survival under metabolic stress. (A) Chemical structure of DHEA. (B) Snail increased NADPH levels and siRNA-mediated or DHEA treatment reverted anti-oxidant level in MCF-7 and MDA-MB-231 cells. (C) Clonogenic survival assay of cancer cells following glucose starvation for 72 h. Knockdown of PFKP increased cell survival under glucose starvation and DHEA (20 μg/ml) treatment reversed the effects of PFKP knockdown. (D) Clonogenic survival of MDA-MB-231 cells against paclitaxel treatment as indicated by concentration. The DHEA and paclitaxel were treated for a 48 h period, and clonogenic capacity was determined. Statistical significances compared with each control are denoted as $^{**}p<0.01$. See Kim *et al.* (2017) for the experimental procedures for NADPH and clononogenic assay.

the X chromosome (Xq28 locus). G6PD deficiency is the most common genetic mutation in the world, estimated to occur in at least 400 million people worldwide (Nkhoma *et al*., 2009). WHO has classified about 300 variants into several classes according to mutant enzymatic activity. Most G6PD deficiencies are class II, having less than 10% activity compared to wild type, although the majority of individuals remain asymptomatic until they are exposed to certain drugs (Stanton, 2012). These suggest that mitochondrial one-carbon metabolism may compensate NADPH during the developmental process and normal life although the contribution of the one-carbon metabolism to NADPH homeostasis in G6PD-deficient individuals is still unknown.

Due to the pivotal role of G6PD in PPP flux and cellular redox balance, there is an interesting epidemiological hypothesis that G6PD deficiency is inversely correlated to cancer incidence and mortality. While lower cancer prevalence in G6PD-deficient individuals was reported from a large epidemiological study conducted in 1980s, the results were inconclusive (Cocco *et al*., 1987). Cancer mortality from all cancer was lower in G6PD-deficient individuals compared to wild-type phenotype (Cocco *et al*., 1996). Recently, a large reduction in colorectal cancer risk among G6PD-deficient individuals was reported in a long-term cohort study of sub-Saharian Africa (Dore *et al*., 2016).

This epidemiological evidence provides important insights for a G6PD therapeutic stategy for human cancer. First, G6PD abundance is increased in many types of human cancer. Furthermore, active oxidative PPP is observed in tumors with multidrug resistance in preexisting chemotherapeutics (Riganti *et al*., 2012). Thus, PPP inhibition may serve as a therapeutic target based on the lower risk of cancer in the G6PD-deficient population. Second, most asymptomatic G6PD-deficient individuals have less than 10% enzymatic activity compared to wild type G6PD. In experimental settings, embryonic stem cells with complete G6PD deletion proliferate normally but are susceptible to oxidative stress (Pandolfi *et al*., 1995; Paglialunga *et al*., 2004). These observations suggest an interesting aspect of PPP inhibition in that a specific inhibitor of G6PD or 6-PGD can be less toxic in normal cells compared to conventional cytotoxic therapeutics. Indeed, a specific inhibitor of 6-PGD effectively attenuates tumor growth *in vivo* without obvious toxicity (Lin *et al*., 2015). However, PPP inhibition alone has limited therapeutic potential because it inevitably increases $NADP^+$, a potent activator of G6PD. There are many G6PD inhibitors (Singh *et al*., 2012; Preuss *et al*., 2013), one of interest being dehydroepiandrosterone (DHEA). DHEA is one of the most abundant circulating endogenous steroids in human, produced from the adrenal gland and gonads during cholesterol metabolism. As an uncompetitive inhibitor of G6PD, DHEA is also used orally as a dietary supplement (Fig. 4A) (Gordon *et al*., 1995). DHEA has very low toxicity in human, daily doses up to 1.6 g taken orally for one month being well tolerated. As mentioned earlier, the Snail/PFKP axis increased PPP flux and NADPH levels in a G6PD-dependent manner and DHEA treatment reversed NADPH homeostasis induced by Snail repressor (Fig. 4B). Since the suppression of PFKP leads to PPP reflux, dynamic knockdown of PFKP significantly increases cancer cell survival under glucose starved condition and DHEA largely attenuates breast cancer cell survival as determined by clonogenic capacity (Fig. 4C). The anti-oxidative capacity is also closely related to chemotherapeutic resistance to paclitaxel, one of the most commonly used therapeutics for human cancer (Ramanathan *et al*., 2005). Indeed, DHEA enhances the therapeutic potential of paclitaxel on MDA-MB-231 breast cancer cells (Fig. 4D). While DHEA clearly inhibits PPP flux and NADPH production *in vitro* (Tian *et al*., 1998; Schafer *et al*., 2009), its anti-cancer effect *in vivo* has not been thoroughly studied. Note that lowered ROS levels are critically required for radioresistance in cancer stem cells (Diehn *et al*., 2009); however, the role of PPP in radiation-induced cancer cell death is also needs further research.

Based on epidemiological evidence of G6PD deficiency and metabolic charateristics of PPP in human cancer, we propose G6PD as a potential target of a synthetic lethal therapeutic approach for human cancer combined with coventional cytotoxic or targeted therapeutics. Other metabolic targets, such as aldehyde dehydrogenase (ALDH), mitochondrial metabolism, glutamine metabolism and fatty acid oxidation, also hold promise for use in combinatory theraputics to inhibit G6PD in cancer (Kim, 2015). Further study of oncongenic function on PPP flux and for development of specific inhibitor of G6PD might provide new insights to clinical benefit of cancer treatment.

CONCLUSIONS

Cancer cells adapt their metabolic circuitry to support proliferation under nourished fast-life as well as to survive under starved slow-life. Emerging evidence suggests that PPP flux is increased in human cancer and that the PPP plays an important role in cancer progression although the oncogenic regulation of PPP is not fully understood yet. During the natural history of cancer initiation and progression, cancer cells are inevitably and repeatedly exposed to a metabolic starved environment. Under starved condition, the ATP and NADPH are essential to the slow-life of cancer cells. The ATP is generated by the oxidation (mainly from oxidative phosphorylation in mitochondria) of many molecules such as glucose, fatty acids, and amino acids. NADPH mainly generated from PPP is the major electron donor in reductive biosynthesis supporting the oxidation process. As discussed earlier, cancer cells have acquired mechanisms to activate PPP based on metabolic demand. Further studies will uncover additional mechanisms by which cancer cells utilize the PPP to support their survival and proliferation. Also, more work is needed to find a novel and specific therapeutics for hyperactive PPP in human cancer.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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