6-phosphogluconate dehydrogenase fuels multiple aspects of cancer cells: from cancer initiation to metastasis and chemoresistance

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Abstract

Reprogrammed metabolism is key biochemical characteristic of malignant cells which represents one of the emerging hallmarks of cancer. Currently, there is rising contemplation on oxidative pentose phosphate pathway (PPP) enzymes as potential therapeutic hits due to their affiliation with tumor metabolism. 6-phosphogluconate dehydrogenase (6PGD), third oxidative decarboxylase of PPP, has received a great deal of attention during recent years due to its critical role in tumorigenesis and redox homeostasis. 6PGD has been reported to overexpress in number of cancer types and its hyperactivation is mediated through post-transcriptional and post-translational modifications by YTH domain family 2 (YTHDF2), Nrf2 (Nuclear factor erythroid 2-related factor 2), EGFR (Epidermal growth factor receptor) and via direct structural interactions with ME1 (malic enzyme 1). Up-regulated expression of 6PGD provides metabolic as well as defensive advantage to cancer cells, thus, promoting their proliferative and metastatic potential. Moreover, enhanced 6PGD expression also performs key role in development of chemoresistance as well as radiation resistance in cancer. This review aims to discuss the historical timeline and cancer-specific role of 6PGD, pharmacological and genetic inhibitors of 6PGD and 6PGD as prognostic biomarker in order to explore its potential for therapeutic interventions. We anticipate that targeting this imperative supplier of NADPH might serve as tempting avenue to combat the deadly disease like cancer.

Keywords: Tumor metabolism; Pentose phosphate pathway; 6PGD; Therapeutic target

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1. Introduction

Cancer is a multifaceted hyperproliferative disorder characterized by genetic mutations, epigenetic modifications, aberrant enzymatic machineries, and erroneous signaling pathways (1, 2). Cancer has emerged as principal health issue and second leading cause of mortalities during recent years with nearly 1670 deaths on daily basis in USA during 2018. The burden of this deadly disease is expected to rise in the years to come (3).

The complex catalogue of cancer biology can be epitomized by six key physiological alterations which are generally familiar as hallmarks of cancer (4, 5). Resisting cellular apoptosis, metabolic rewiring, induction of angiogenesis, escaping anti-growth signals, limitless replicative capacity, and initiating invasion and metastasis represent acquired properties of cancerous cells (6, 7). These six biological capabilities enable cancer cells to become the master of their own fate.

Recently, metabolic rewiring has been reported as emerging hallmark of cancer (8). Rewired metabolism refers to up-regulated metabolic flux which is attained by adaptations in multiple metabolic and signaling pathways (9, 10). One of the key characteristic of this altered metabolic profile is dependence of cancer cells on glycolytic pathway instead of more energetically favorable oxidative phosphorylation (11). The matter of subject here is why cancer cells adopt these energetically less efficient pathways? Taking into account of complexity of metabolic pathways, it becomes obvious that these reprogrammed pathways unitedly drive malignant behavior of cancer cells by providing not only energy (ATP) but structural units for macromolecule synthesis (amino acids, nucleotides and sugars), redox regulators (NADPH), and reducing powers (12, 13). The requirement for these biosynthetic intermediates to fuel the proliferation of cancer cells could explain why cancerous cells fluctuate towards augmented glucose uptake. These demands of tumors are fulfilled by various pathways branching off from core glycolytic cascade (14) More than 20 pathways of carbohydrate, amino acid and lipid metabolism have been reported to overexpress in cancer cells (15).

Pentose phosphate pathway (PPP) branches off from glycolytic cascade at the first step. PPP pathway serves as a major source of NADPH and structural units for cancer cells (16). Multitudinal lines of evidences support the key role of NADPH homeostasis in survival of cancer cells under metabolically and ROS-mediated stressed circumstances. Thus, rewiring of NADPH

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homeostasis in ROS-induced oxidative stressed cancer cells is an auspicious approach in cancer therapeutics (17, 18).

6-Phosphogluconate dehydrogenase (6PGD) catalyzes the conversion of 6-phosphogluconate (6-PG) into ribulose 5-phosphate (R-5-P) in the third step of PPP, thereby, producing nicotinamide adenine dinucleotide phosphate (NADPH) (19). Up-regulation of 6PGD has been reported in multiple human cancers (20). Thus, targeting 6PGD might serve as tempting avenue to combat the deadly disease like cancer.

This review is the first attempt to summarize the historical timeline, characteristics and role of 6PGD in cancer to provide new insights into this enzyme. In addition, we have also highlighted the role of 6PGD in metastasis, chemoresistance, pharmacological inhibitors, 6PGD as biomarker for cancer along with future directions.

2. Historical Perspective of 6PGD

This section aims to shed light on historical perspectives and milestones in the study of 6PGD from 1920 to till date. The data is compiled from scientific researches that have shaped our views on the current understanding of 6PGD role in cancer in the last 100 years (Figure 1). The story of modern cancer research on tumor metabolism started from the early 19^{th} century with the ground breaking observations of Otto Warburg about differences in glucose metabolism of normal and tumor cells (21). Warburg hypothesized augmented glucose uptake and overexpressed glycolytic pathway as key characteristics of cancer cells. In 1930s, Warburg discovered the existence of another oxidative pathway for the metabolism of glucose 6-phosphate (G6P) in addition to glycolytic cascade. The key evidence supporting this mechanism was the utilization of NADP+ for the oxidation of G6P, which was later reported as the first step of the PPP (22). However, in the 1950s, it was the central work of Dickens and Lipmann that elucidated the PPP entirely (23) and the first complete draft of PPP was presented in 1955 (24). These studies undoubtedly served as foundation for the future emerging trends of tumor metabolism. However, nearly half of a century passed before the onset of tumor metabolism as emerging hallmark of cancer. In 1962, Bonham and Gibbs published a preliminary report on 6PGD-based biochemical assay for the early diagnosis of cervical cancer. However, the sensitivity and specificity of the assay was questioned by several researchers which led to the modifications in the purposed assay in 1967

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(25). Till 1968, isolation of 6PGD from several non-mammalian resources such as brewers' yeast, *Escherichia coli*, *Candida utilis* has been reported (26).

While finding out the relationship between glucose-6-phosphate dehydrogenase (G6PD) deficiency and schizophrenia, a female patient was found to exhibit deficiency of another erythrocytic enzyme, 6PGD in 1964 (27) which was later found to be extremely rare enzymopathy in humans and only associated with slight hemolysis (28).

Although isolation of 6PGD from rat liver was reported in 1953 (29), however, 6PGD was firstly purified from mammalian tissues in 1969 from sheep liver by Villet and Dalziel (30). In 1977, gradual rise of 6PGD activities in highly metastatic grade III breast cancer tissues was observed (31). The role of 6PGD in development and progression of cancer emerged during the second decade of 20^{th} century after the discovery of the role of 6PGD in regulation of p53-mediated senescence induction in lung cancer cells (32). First post-translational modification of 6PGD by acetylation at K76 and K294 was identified in 2014 (33) while later in 2019 phosphorylation of 6PGD at tyrosine 481 (34) and post-transcriptional modification of 6PGD mRNA known as m6A was reported (35). These modifications are responsible for the regulation of 6PGD by promoting its expression, activity and mRNA translation. Interestingly in 2015, first 6PGD inhibitor, physcion and its derivative S3 were identified and reported to have potential against tumor growth in cancer xenografts without any obvious toxicity (20). In 2017, 6PGD was first reported to be involved in development of cisplatin resistance in lung and ovarian cancer (36) and later other studies showed that 6PGD is involved in development of resistance against chemo-drugs (37). Researchers recently explored and presented that 6PGD also promote radiation resistance in brain cancer cells (34). From the trajectory of this history, we speculate that 6PGD could evolve towards novel therapeutic drug target for cancer by further exploitation and future discoveries in this field.

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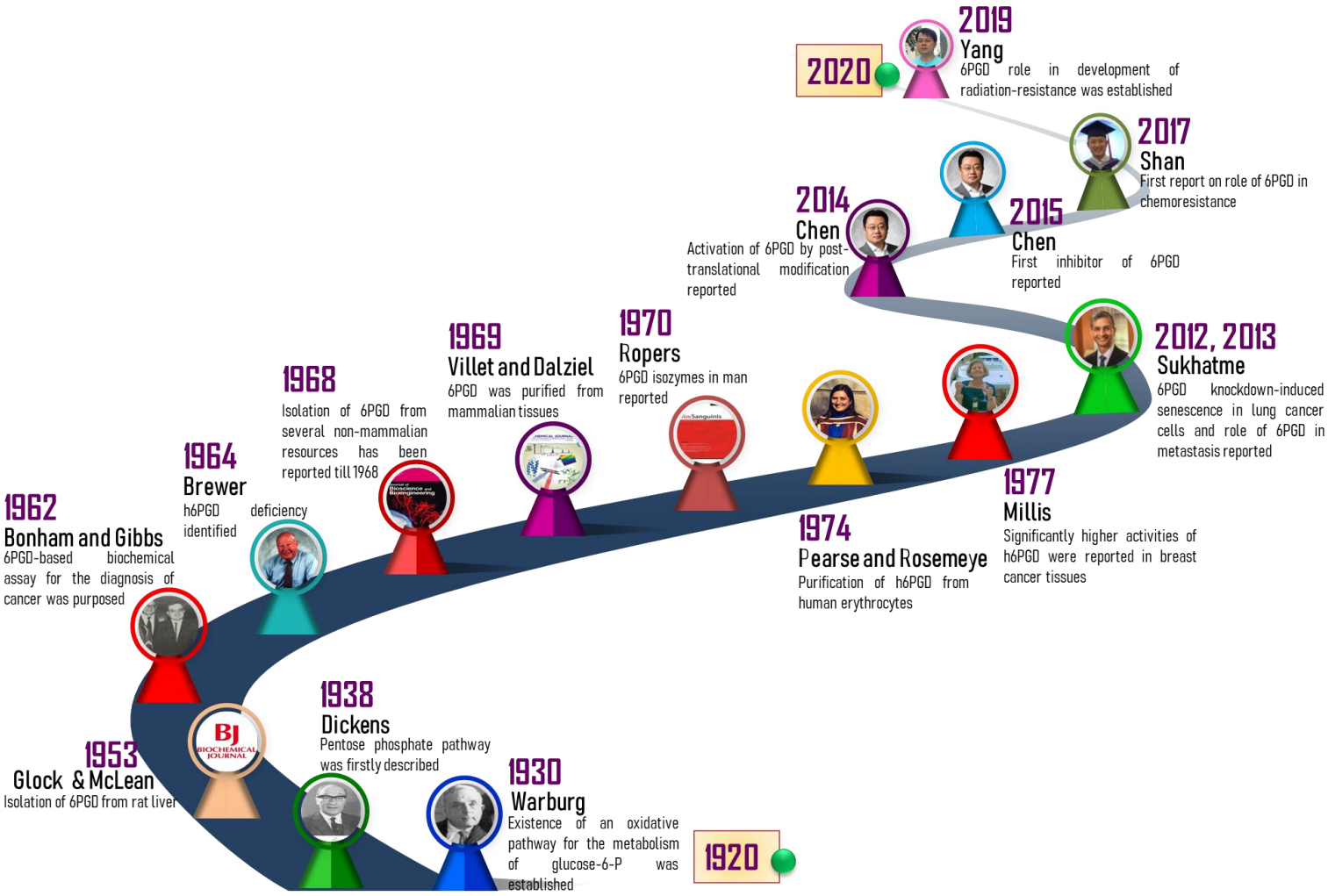

Figure 1. A timeline of key events in the study of 6PGD

3. Characteristics of 6PGD

6PGD is a homodimer protein whose monomers act independently to each other. Each monomer consists of large alpha-helical domain while small beta-alpha-beta domain, forming a 6 stranded beta sheet. The enzyme is reported to be NADP-dependent (38). NADP binding site is in a cleft of the small domain while the substrate binding site is found in an adjacent pocket. Genetic evidences specify the locus of the 6PGD gene on chromosome 1p (39). Cellular fractionation studies have indicated the location of the enzyme encoded by 6PGD gene almost entirely in the cytosol (40). The 6PGD protein consists of 468 residues in *E. coli* and 482 residues in sheep while 88 residues were found to be conserved in nearly all sequences (41).

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From tissues of humans, 6PGD was first isolated from erythrocytes followed by purification from granulocytes and then from human brain (42). The full length clone of h6PGD encodes 483 amino acids long open reading frame. Aligned amino acid sequences of sheep 6PGD and h6PGD displayed 94.2% identity between proteins. The molecular weight of h6PGD has been reported as 53,149 daltons. The secondary structure of h6PGD encompasses 36% alpha-helix while 9% beta-sheets (43).

4. Role of 6PGD in cancer progression

In order to meet the high energy demands, cancerous cells adapt a path of rewired metabolism. More than 20 metabolic pathways have been reported to be reprogrammed in cancer cells (15). Metabolic reprogramming of cancer cells is characterized by overexpression of metabolic enzymes. Overexpression of 6PGD in multiple cancers has caught researcher's attention in recent years (Figure 2). Nevertheless, the mechanism by which 6PGD promotes cancer progression is still under investigation. However, the probable answers behind the tumor progressive roles of 6PGD might lies in its capability to mediate multiple biological functions such as lipogenesis, redox homeostasis (20), metastasis (44) and proliferation of cells (37).

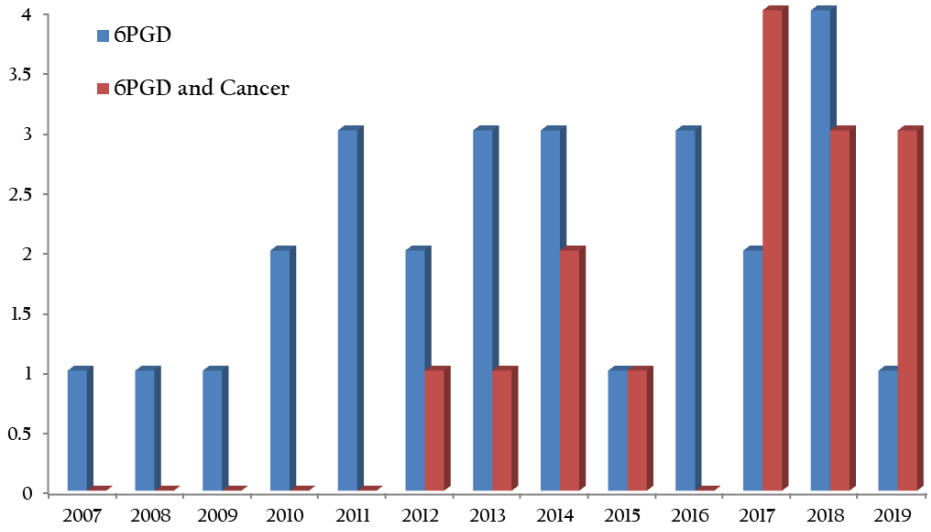

Figure 2. 6PGD-related publications since 2007. Data was collected from PubMed

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Foregoing researches have reported up-regulation of 6PGD in multiple human cancers such as colorectal tumors (39), breast carcinoma (45), hepatocellular cancer (37), thyroid cancer (46), cervical neoplasia (47), leukemia (20), ovarian (48) and lung carcinoma (32) (Figure 3). Cancer is complex disease encompassing >200 different types and many of them have multiple subtypes. However, the role of 6PGD has been investigated in eight major types of cancer till now. Moreover, whether overexpression of 6PGD is a general rule or it varies from cancer to cancer also need to be investigated. Therefore, it would to worthwhile to examine the expression and activity of 6PGD in other types of cancer.

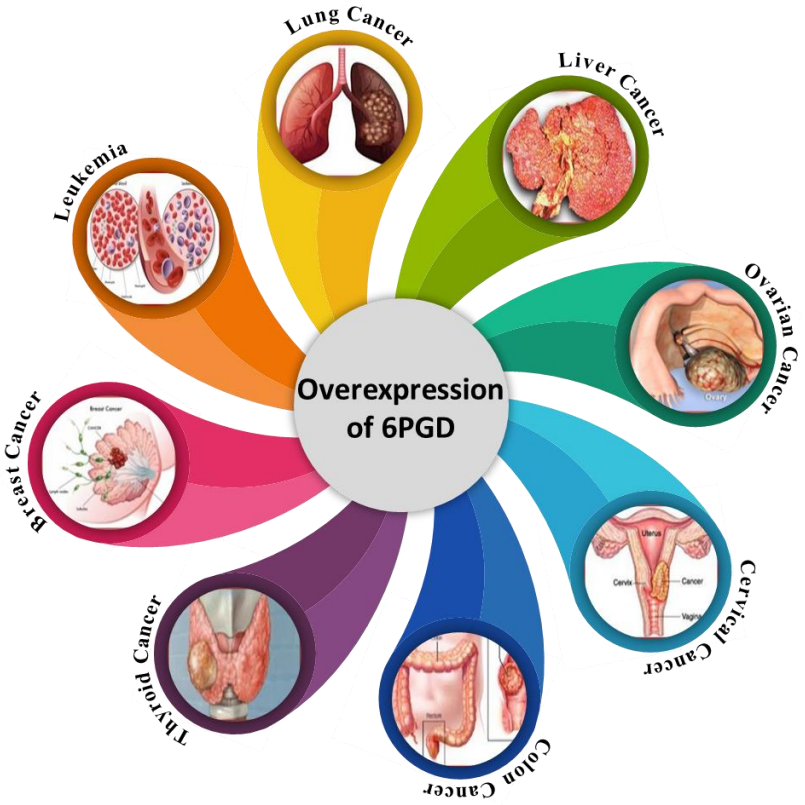

Figure 3. Overexpression of 6PGD in different cancers

4.1 How 6PGD gets activated?

To figure out the role of metabolic transformations in cancer, we must firstly understand the regulatory circuits which lead to the hyperactivation of metabolic enzymes. Therefore, this section aims to decipher that how 6PGD gets activated in cancer cells?

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4.1.1 YTH domain family 2 (YTHDF2)-facilitates 6PGD mRNA translation by binding to m6A

N6-methyladenosin (m6A) mRNA modifications and its associated proteins (writers, readers and erasers) have been emerged as important players of cancer development and progression in recent years (49). mRNA modification associated readers and writers overexpress in number of cancer types. Methyltransferases (writers) such as METTL3 catalyze the transfer of methyl group to mRNA, thus, leading to formation of modified m6A mRNA (50). m6A regulates the translation and stability of proteins by recruiting reader proteins such as YTHDF2. YTHDF2 selectively recognize m6A for binding and affects mRNA lifetime as well as mRNA translational potential (51). It has been reported recently that YTHDF2 is an important regulator of 6PGD expression. YTHDF2 promotes 6PGD mRNA translation by directly binding to m6A modification site in lung cancer cells [Figure 4 (a)], thus, promoting metastasis and tumor growth of lung cancer (51).

4.1.2 Activation of 6PGD by EGFR-mediated phosphorylation

Overexpression of epidermal growth factor receptor (EGFR) has been reported in 40% gliomas. While in 50% of cancers, EGFR amplification has been documented (52). EGFR has potential to reprogram metabolic profile of tumors to derive cancer progression as well as resistance to anti-cancer drugs. But how EGFR reprogram tumor metabolism? Recently it has been reported that activated EGRF promotes 6PGD phosphorylation by Fyn, a Src family kinase, at tyrosine Y481. Upon phosphorylation, binding interaction of 6PGD to NADP+ is up-regulated [Figure 4 (b)], thus, leading to the increased NADPH and R-5-P intracellular levels (34). NADPH leads towards the detoxification of ROS (53) and R-5-P enhances DNA synthesis (54). These cascades of molecular events provide metabolic as well as defensive advantage to cancerous cells by promoting their proliferative potential. Whether all EGFR-dependent cancers overexpress 6PGD or not should be explored in future studies.

4.1.3 Overexpression of 6PGD via acetyltransferases-induced acetylation

Acetylated 6PGD at lysine residues has been reported in human cancerous cells. Acetylation of 6PGD at K294 and K76 site promotes the formation of active 6PGD dimers. Moreover, it has been reported that ACAT2 (acetyl-CoA acetyltransferase 2) and DLAT (dihydrolipoamide S-

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acetyltransferase) serve as acetyltransferases of K294 and K76 site, thus, causing acetylation of lysine residue of 6PGD [Figure 4 (c)]. Treatment of K562, H1229, MDA-MB-231, 212LN (leukemia, lung, breast, neck cancer cells respectively) cells with deacetylase inhibitors led to increased endogenous 6PGD levels which further validated that acetylation promotes 6PGD activation to derive tumor progression and provide metabolic advantage to cancerous cells (33). However, whether ACAT2 and DLAT directly cause the acetylation of 6PGD or recruit other acetyltransferases for this purpose need to be explored in future studies.

4.1.4. 6PGD activation via interaction with ME1 (Malic enzyme 1)

Crosstalk between PPP and ME1 via direct structural interactions promotes PPP flux, NADPH production and tumor growth. Overexpression of ME1 has been reported to upregulate PPP flux via increasing the enzymatic expression and activity of 6PGD in U2OS and A549 cells. A hetero-oligomer is formed by structural interactions between ME1 and 6PGD leading towards to activation of 6PGD [Figure 4 (d)]. Activated 6PGD has enhanced capability to get bind to its substrate and thus production of intracellular NADPH is up-regulated (55). The results from this study have unveiled the crosstalk between two NADPH producing enzymes (ME1 and 6PGD) and there are several NADPH producing enzymes of metabolic pathways. Thus, it would also be worthwhile to investigate the coordination networks of other NADPH- producing enzymes in order to understand the perplexing nature of tumor metabolism.

4.1.5 Nrf2-mediated activation of 6PGD

Nrf2 (Nuclear factor erythroid 2-related factor 2), a master transcriptional factor, is responsible for the activation of cyto-protective genes. Stabilization and translocation of Nrf2 to the nucleus and its binding to antioxidant response element (ARE) activates various genes encoding anti-oxidant proteins (56). Overexpression of Nrf2 is responsible for the poor prognosis of cancer due to aggressive nature of tumors. Nrf2 directs metabolic reprogramming by directly activating 6PGD via well-conserved AREs [Figure 4 (e)] leading to the acceleration of tumor growth in A549 cells (57). PKM2 (Pyruvate kinase M2) has been known to activate STAT3 (Signal transducer and activator of transcription 3) (58) and Nrf2 activates 6PGD. Thus, interactions between metabolic enzymes and transcriptional factors might be an imperative factor for

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development of cancer. Therefore, targeting the interactions of metabolic enzymes and transcriptional factors could be a novel approach to treat cancer.

4.1.6 Crosstalk between PGAM1 (phosphoglycerate mutase 1) and 6PGD

Knockdown of glycolytic enzyme, PGAM1 results in up-regulation of its substrate 3PG (3-phosphoglycerate). 3PG binds to 6PGD and inhibits its activity which clearly depicts that PGAM1 inhibition leads to the inhibition of 6PGD activity (12, 59). However, there is no direct evidence reporting the role of PGAM1 up-regulation in activation of 6PGD which need to be investigated by researchers.

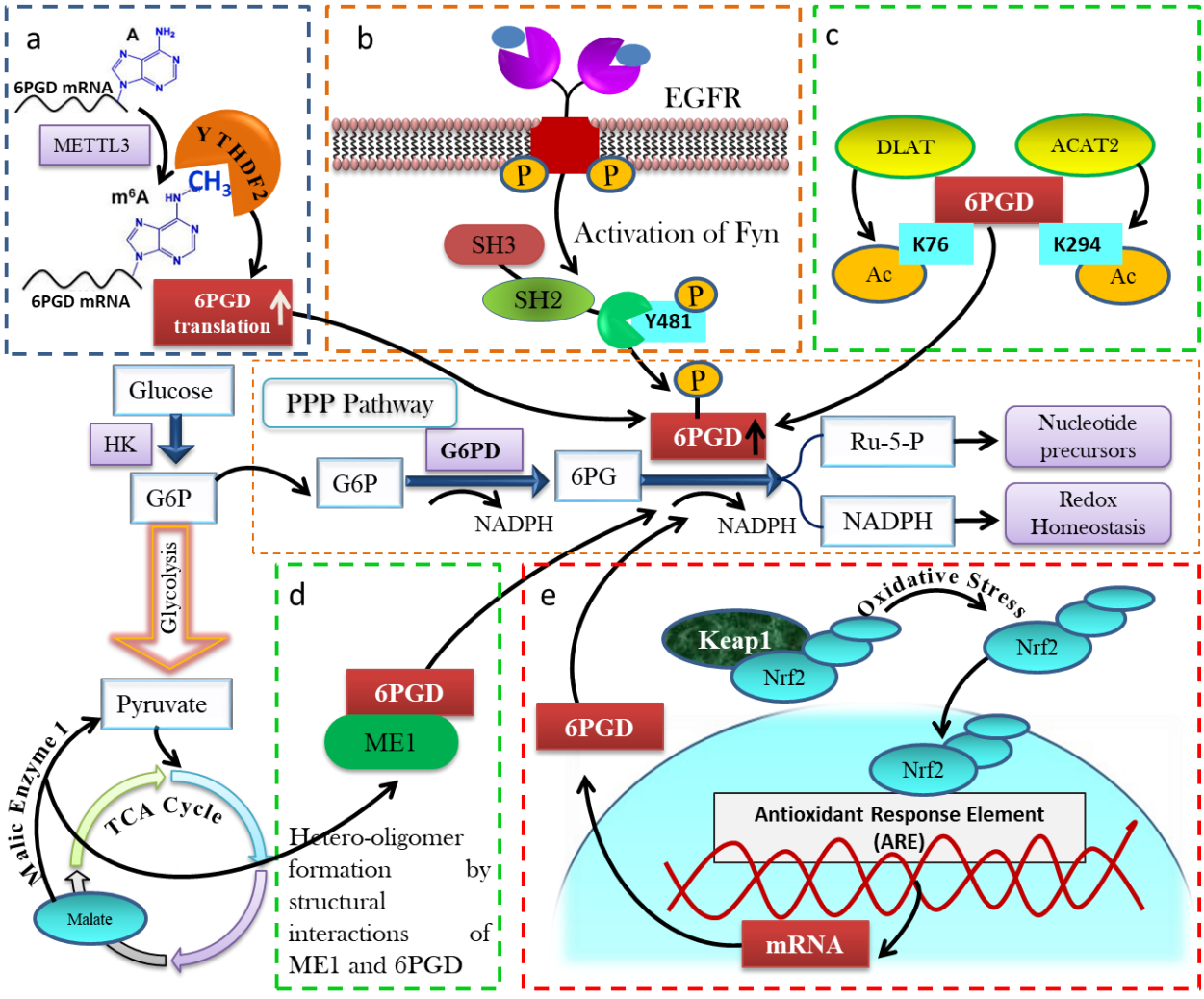

Figure 4. How 6PGD gets activated? Crosstalk of 6PGD with other signaling pathways. (a) Binding of YTHDF2 to the m6A modification site of 6PGD mRNA facilitates 6PGD mRNA

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translation (b) Activation of Fyn by phosphorylation activates 6PGD, (c) structural interactions with ME1 enhances the binding potential of 6PGD to its substrate, (d) Acetyltransferases-mediated acetylation leads to the hyperactivation of 6PGD, (e) Transcriptional regulation by Nrf2 enhances the expression and activity of 6PGD

4.2 Tumor initiation and metastatic transformations via 6PGD hyperactivation

6PGD expression is directly correlated with tumor progression and metastatic abilities of cancerous cells (Table 1, Figure 5). The tumor suppressor liver kinase B1 (LKB1) serves as an up-stream kinase of AMPK (AMP-activated protein kinase) pathway. LKB1-AMPK performs principal role in mediating cellular proliferation, cell survival and metabolism to promote cellular survival in response to energy-stressed conditions (60, 61). Knockdown of 6PGD leads towards reduced levels of Ru-5-P below its physiological proportion which successively not only attenuated biosynthesis of nucleotides but also relieved the prohibition of LKB1, thus, activating AMPK with consequent inhibition of lipogenesis. The results from this study recommend that 6PGD knockdown decreased lipogenesis via Ru-5-P-mediated prohibition of LKB1-AMPK signaling and up-regulated ROS levels, thus, attenuating proliferation and tumor growth (20).

Inhibition of 6PGD has potential to activate AMPK along with its down-stream substrate ACC1 causing decreased ACC1 activity which ultimately reduces biosynthesis of lipids in breast cancer cells (Figure 5) (45). Overexpression of 6PGD in hepatic cancer cells could activate AMPK-mediated NADPH metabolic reprogramming, thus, conferring metabolic advantage to cancer cells (37). In addition to AMPK activation in lung, leukemia and breast cancers, 6PGD also targets AMPK in cervical cancer. Further, 6PGD has also potential to target Rac1 and RhoA in cervical cancer cells. Rac1 and RhoA are Rho GTPases which derive lamellipodium, lobopodium, fibroblasts and bleb-mediated migration in cancer cells (62). 6PGD inhibition causes a marked reduction in the expression of Rac1 and RhoA which ultimately reduced invasive capabilities of cancer cells. However, 6PGD inhibitors were unable to reduce Rac1 and RhoA expression in AMPK depleted cells which clearly declare that 6PGD mediate Rac1 and RhoA expression in an AMPK-dependent manner (63).

In another attempt to understand 6PGD-regulated networks, it was found that 6PGD appears to be essential for activation of c-Met by increasing its phosphorylation at tyrosine residues in lung

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cancer cells (44). As c-Met activation have potential to derive proliferation, detachment of epithelial cells and their motility (64, 65). Therefore, activation of c-Met by 6PGD might be imperative factor for 6PGD derived metastasis of lung cancer cells. As 6PGD expression has been found to be positively correlated with advancing metastatic stage of lung carcinoma tissues (66).

Overexpression of 6PGD mediates the expression of p53 leading towards the suppression of senescence (Figure 5). Induction of senescence oppose neoplastic transformations which itself is obstructed by metabolic reprogramming or oncogenic mutations. p53 is most imperative regulator of senescence which is mostly inactivated or mutated in various cancers (67, 68). 6PGD knockdown up-regulated the expression of p53 and its downstream target p21Waf1/Cip1/Sdi1 in H1975 lung cancer cells, thus, induced cellular senescence and rescued cells from neoplastic transformations (32). Further experimentations are surely needed to provide a complete picture of 6PGD-mediated signaling networks, especially those which are associated with senescence suppression and metastasis of cancerous cells.

4.3 NADPH producing enzymes as redox stabilizers

Altered metabolic profile and stress responses have been recently reported as emerging hallmarks of cancer (69, 70). Multiple lines of evidences have illustrated that cancer cells are under oxidative stress due to increased levels of ROS (71). In order to neutralize the ROS-induced stress, cancer cells become dependent on ROS stabilizing systems such as glutathione (72). Thus, targeting such ROS scavengers have potential to selectively kill cancer cells by shifting redox balance towards oxidative stressed conditions (73). For the maintenance of redox homeostasis, reduced glutathione (GSH) is required and the recycling of oxidized glutathione (GSSG) to reduced GSH is dependent upon reducing equivalent such as NADPH (74). Therefore, enzymes that are responsible for generating NADPH including G6PD (75), 6PGD (76) and ME2 (7) promote cancer cell survival under stress. Convincing evidences have demonstrated that cancer cells trigger the overexpression of NADPH generating enzymes which is a result of metabolic reprogramming (77).

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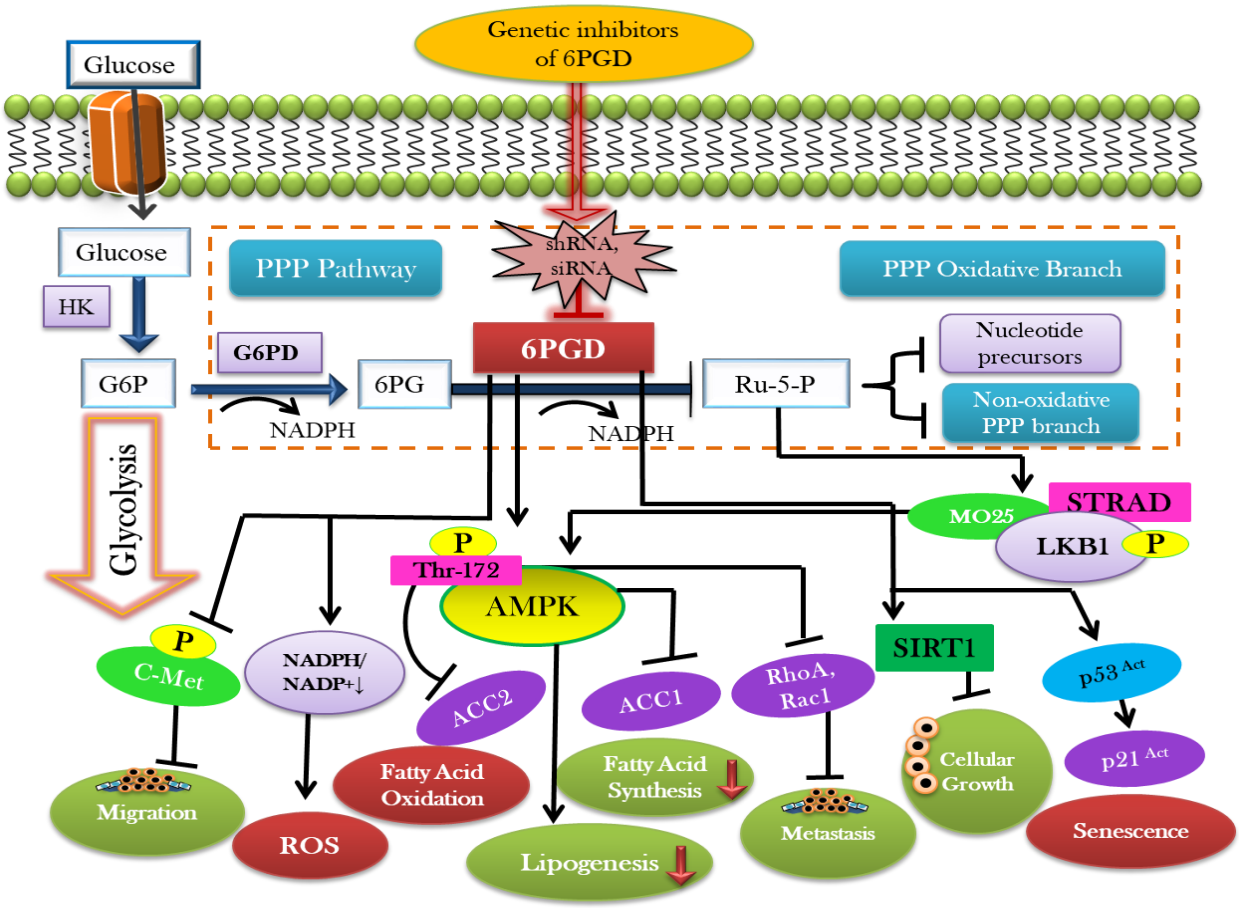

Figure 5. Genetic inhibitors of 6PGD block the overexpression and activity of 6PGD resulting in inhibition of metastasis, cellular growth and lipogenesis and induction of senescence in cancer cells.

4.4 6PGD and chemoresistance

Interestingly, aberrant expression of 6PGD has also potential to induce chemoresistance in lung, breast, liver and ovarian cancer. Exposure of control and 6PGD-depleted MCF-7 and MDA-MB-231 cells to paclitaxel and doxorubicin chemotherapeutic agents has declared that 6PGD-depleted cells are more sensitive towards anti-cancer agents. 6PGD-depleted cells show reduced viability and proliferative potential as compared to control in response to anti-cancer drugs (45).

In addition, established cisplatin-resistant lung (A549DDP) and ovarian (C13*) cancer cells showed higher expression of 6PGD when compared to their cisplatin sensitive counterparts (A549 and OV2008). It was observed that up-regulated 6PGD in cisplatin-resistant cells is correlated to reduced expression of miR-613 and miR-206 that are reported to target 6PGD.

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Stable knockdown of 6PGD or its inhibition by pharmacological agents have potential to enhance sensitivity of cisplatin-resistant cells towards cisplatin (36). Persistently, inhibition of 6PGD not only sensitizes ovarian and lung cancer cells towards cisplatin but also towards anti-malarial drug, dihydro-artemisinin (79). Depletion of 6PGD by genetic inhibitors markedly restored the efficacy of chemo-drugs in HCC cells as evident by effective inhibition of proliferation and apoptosis induction (37). Doxorubicin resistant anaplastic thyroid cancer cells (ATC) aberrantly overexpress mRNA and protein of 6PGD. Pharmacological and genetic inhibition of 6PGD has tendency to disrupt the metabolic reprogramming of thyroid cancerous cells which increase their sensitivity towards doxorubicin (78). Thus, targeting 6PGD could improve the therapeutic potency of chemo-drugs such as cisplatin, doxorubicin and paclitaxel which could serve to defeat chemo-drugs resistance. Moreover, rational combination of 6PGD inhibitors along with chemo-drugs can provide an efficacious combinatorial treatment against cancer.

Table 1. Status and role of 6PGD in various cancers

Cancer type	Status of 6PGD	Role of 6PGD in cancer	References
Lung	Overexpressed	Derives migration of cancerous cells via c-Met activation, development of chemoresistance	(44)
Breast	Increased transcriptional and translational levels with enhanced enzymatic activity	Mediate lipid biosynthesis via modulation of AMPK signaling and its down-stream substrate ACC1 and ACC2	(45)
Liver	Overexpression and increased enzymatic activity	Modulation of oxidative stress via alteration of $NADPH/NAD^+$ ratio and SIRT1 expression	(37)
Thyroid	Up-regulated mRNA, protein and enzymatic activity	Development of chemoresistance	(78)

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Cervical	Up-regulated mRNA, protein and enzymatic activity	Stimulation of Rac1 and RhoA-mediated migration and invasion of cancer cells	(63)
Leukemia	Increased enzymatic activity	Mediate RNA and lipid biosynthesis as well as ROS production via targeting LKB1-AMPK signalling	(20)
Ovarian	Overexpression and increased enzymatic activity	Development of chemoresistance	(36)
Colorectal	Overexpressed		(39)

5. 6PGD as a potential therapeutic drug target for cancer

6PGD knockdown in H1975 and H1650 lung cancer cells by shRNA system reduced the proliferation of cancerous cells. However, overexpression of 6PGD in 6PGD knockdown cells restored the proliferative capability of cancerous cells. Moreover, 6PGD knockdown by shRNA in doxycycline-induced xenografted tumor mouse model significantly halted the growth of tumors (32). Knockdown of 6PGD in H1299 cells leads towards decreased oxidative PPP flux, reduced ratio of NADPH/$NADP^+$ and increased activity of phosphofructokinase from glycolytic cascade, thus, attenuating the proliferation of cancer cells (20). Inhibition of 6PGD increased sensitivity of cancer towards chemotherapeutics in *in vitro* as well as *in vivo* xenografted breast cancer model (45). 6PGD knockdown in HI975 cells inhibited invasive capabilities of cancerous cells *in vitro* (44). Functional inhibition of 6-PGD by genetic and pharmacological inhibitors halted the growth and reduced survival via induction of apoptosis in hepatocellular cancerous cells (37). Pharmacological inhibition of 6PGD in cervical cancer cells significantly prohibited Rho-GTPases mediated migration of cancer cells (63). Partial inhibition of 6PGD enzyme (36-61% of control) was enough to diminish NADPH supply (58-61% of control) and reduce the growth to 39-44% of control leukemia cells suggesting that 6PGD serve as a principal mediator of cancer development (80). Inhibition of 6PGD expression induced p53-dependent senescence in H1975 cells which rescued cells from neoplastic transformations (32). All these findings

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cumulatively suggest that 6PGD might serve as promising therapeutic target for the treatment of cancers in which 6PGD is aberrantly overexpressed.

5.1 Pharmacological inhibitors of 6PGD

Upregulated oxidative PPP with constitutive overexpression of 6PGD correspondingly accelerates energy supply, production of nucleotides precursors as well as ROS scavengers. Therefore, 6PGD inhibition provides dual functional strategy such as reduced obligatory energy supply with blockage of mandatory anabolic processes and disruption of redox homeostasis to reduce cell proliferation as well as tumor growth.

In vitro enzymatic assay based screening strategy has identified physcion as 6PGD inhibitor from library of 2,000 FDA approved small molecules. Furthermore, a derivative of physcion known as S3 was also identified as 6PGD inhibitor with more potency (Figure 6). Physcion and S3 has potential to inhibit 6PGD (IC_{50} = 38.5 μM and 17.8 μM, respectively) leading to decreased proliferative potential of K562 and H1299 cells. Physcion's mechanism of inhibition proceeds via fitting in the pocket of 6PGD surrounded by K261, K71, M15 and H452 residue which is proximal to the binding site of 6PG. Physcion interacts hydrophobically with the residues and forms hydrogen bonding through its 10-keto group with N103, thus, inhibiting its activity (20). Although it has been reported that Physcion and S3 inhibited the growth of K562 and HI299 cells *in vitro* and tumor growth *in vivo*, further studies should also focus on the cytotoxic effect of these inhibitors against other cancers along with toxicological profiles of these inhibitors.

In another attempt to develop and characterize 6PGD inhibitors, N-substituted indole derivatives of N-benzoylindoylbarbituric acid and indomethacin were screened to test their inhibitory potential against 6PGD. N-Benzoylindole, N-(4-Chlorobenzoyl) indole, and 1H-Indol-1-il)(tiyofen-2-il)methanon were found to be potent inhibitors of 6PGD (IC_{50} = 2.75 μM, 2.19 μM, 3.36 μM respectively) (Figure 6). N-Benzoylindole inhibited 6PGD non-competitively while other two were characterized as competitive inhibitors of 6PGD (81). No one has still attempted to explore the cytotoxic activity of these inhibitors against different cell lines which should be investigated. Moreover, biosafety profiles of 6PGD inhibitors are also needed to be explored. As 6PGD has been reported as a potential therapeutic drug target and only few 6PGD inhibitors

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have been identified still, thus, additional investigations should focus on the identification and development of potent and selective 6PGD inhibitors which will surely find their clinical applications for the treatment of 6PGD-dependent cancers.

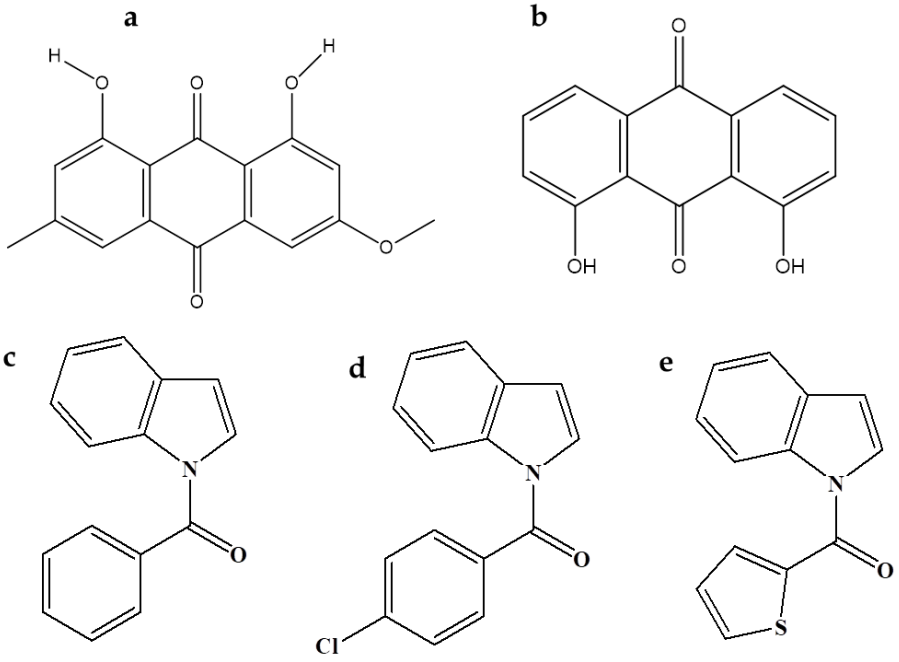

Figure 6. Structural representation of pharmacological inhibitors of 6PGD. **(a)** Chemical structure of Physcion, **(b)** Chemical structure of S3**, (c)** Chemical structure of N-Benzoylindole, **(d)** Chemical structure of N- (4-Chlorobenzoyl) indole, **(e)** Chemical structure of 1H-Indol-1-il)(tiyofen-2-il) methanon.

6. 6PGD as a prognostic biomarker

Overexpression of 6PGD has been reported in all seven lung cancer tissue samples (Papillary adeno carcinoma, small cell/ large cell carcinoma, adeno squamous and squamous cell carcinoma's) as compared to the normal tissue of lungs (32). Another investigation has also documented the low expression of 6PGD in normal lung tissues while overexpression of 6PGD has been found to be positively correlated with advancing stage of lung carcinoma tissues (44). Thus, 6PGD might serve as a prognostic biomarker for lung cancer tissues.

Aberrant activation of 6PGD was also found in breast cancer tissues as evidenced by its enhanced transcriptional as well as translational levels along with its enzymatic activity in breast

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cancer cell lines and tissues when compared to its normal counterparts (45). Patients of primary breast cancer with high 6PGD activity have poor relapse-free survival time as compared to patients with low 6PGD activities ensuring the value of 6PGD expression as prognostic biomarker (48). 6PGD activity was 1.5 to 4 folds up-regulated in tissues obtained from hepatocellular carcinoma patients when matched with normal liver tissues (37). 6PGD protein expression in cervical cancer tissues was also reported to be significantly high as compared to normal cervical tissues (63). Although various studies have reported overexpression of 6PGD in several cancer types, however, further investigations are surely needed to define it more carefully that how 6PGD expression and activity is correlated to metastasis status, carcinoma types and tumor grade in cancers. It would also be interesting to determine the level of 6PGD in serum and blood of normal and cancer patient samples for the possible utilization of 6PGD as diagnostic biomarker.

7. Conclusions and future perspectives

Latest researches have revealed this novel multi-faceted biological molecule, 6PGD, as an emerging therapeutic target for cancer. Multiple lines of evidences support the fact that 6PGD enhances the proliferation, survival and metastasis of tumor cells via reprogramed tumor bioenergetics. Moreover, 6PGD overexpression contributes towards the development of chemoresistance. However, no one has attempted to uncover the mechanism associated with chemo-drug resistance. Whether EGFR- mediated increased phosphorylation of 6PGD contribute towards chemoresistance? Or 6PGD-mediated modulated expression of p53 derives resistance development in cancer cells? Which should be investigated in future studies. Furthermore, non-metabolic/non-enzymatic functions of 6PGD along with protein-protein interactions of normal 6PGD function should be focus of future researches in order to design and assure the efficacy of novel inhibitors of 6PGD. Furthermore, how crucial are these non-metabolic/non-enzymatic functions of 6PGD for normal cellular processes also needed to be determined? As PGAM1 has been reported to interact with 6PGD, thus, crosstalk between these metabolic pathways also needs consideration. It will also be worthwhile to explore biosafety profiles and side effects of the available 6PGD inhibitors along with their metabolic-dependent toxicity. These future investigations are surely needed to turn the therapeutic targeting of 6PGD into a reality. Thus, we

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anticipate future opportunities in providing the complete picture of 6PGD in cancer progression to translate these findings into preclinical and clinical aspects.

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