Exploring the role of glucose-6-phosphate dehydrogenase in cancer (Review)

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Abstract. Glucose-6-phosphate dehydrogenase (G6PD) is a cytoplasmic enzyme found in human erythrocytes that provides reduced NADPH for cell metabolism. Glutathione produced by the G6PD pathway can reduce the degree of harm caused by reactive oxygen species such as oxygen-containing free radicals, peroxides and lipid peroxides. Investigation of G6PD has long focused on hemolysis, jaundice and other diseases caused by defects in its function. However, increased mRNA expression levels of G6PD are predictive of adverse clinical outcomes in cancer patients, including increased drug resistance, migration or proliferation of tumor cells. Mutations in the G6PD gene affect protein expression and activity, and alters the balance of redox states, leading to disease. However, the association between G6PD and tumors is incompletely understood. The aim of the present review was to summarize the current body of knowledge on the role of G6PD in tumor progression and the possible regulatory mechanisms involved. It is hypothesized that G6PD will prove to be of value as a target of cancer treatment in the near future.

Contents

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1. Introduction

Glucose-6-phosphate dehydrogenase (G6PD) is conventionally considered as the first and rate-limiting enzyme of the pentose phosphate pathway (PPP), is present in the cytoplasm of red blood cells and protects cells against oxidative damage (1). PPP produces large quantities of NADPH and ribose 5-phosphate for various cellular synthetic functions, such as the synthesis of aliphatic acid and sterols. In addition, this pathway ensures glutathione (GSH) reduction, which enhances antioxidant defense and promotes cell proliferation (2). Individuals with clinical G6PD deficiency are prone to neonatal jaundice, infection or drug hemolysis, chronic non-spherical red blood cell hemolytic anemia and abnormal lipid metabolism (3). Furthermore, mutations in G6PD have been previously demonstrated to be the primary cause of certain diseases, such as chronic hemolytic anemia (4).

It has been shown that the expression of G6PD in tumor cells is higher compared with that in normal cells, and its expression is associated with the overall survival of tumor patients (2,5). Numerous studies have also demonstrated increased G6PD activity in several types of cancer, including bladder cancer, carcinoma of the endometrium, prostate cancer, kidney cancer, stomach cancer, cholangiocarcinoma, colon adenocarcinoma, lung cancer, cervical cancer, carcinoma of the ovary, hepatocellular carcinoma (HCC), glioma, pancreatic cancer and melanoma (6-17). Based on the findings of previous studies, the aim of the present review was to focus on the mechanism underlying the role of G6PD in tumorigenesis and tumor development, as G6PD is hypothesized to become a topic of increased interest with regards to cancer in the future.

2. Biological function of G6PD in normal cells

G6PD is encoded by the G6PD gene, which is localized on chromosome X in the high-density region xq28, and is 18 kb in length, consisting of 13 exons and 12 introns. G6PD exhibits significant genetic diversity and the full-length cDNA of the human G6PD is 1,548 bp. The quaternary structure of G6PD is present as a dimer of two identical monomers (18), and the active form is a dimer or tetramer. Each monomer contains 514 amino acids in humans, and has a catalytic coenzyme-binding site and a substrate-binding site that binds to

glucose-6-phosphate (G6P) (19,20). G6PD is a housekeeping enzyme and is present in all tissues and organs. To the best of our knowledge, the first description of the physiological role and effects of G6PD were presented in 1931 (21). To date, >400 biochemical variants and >200 genetic variants of G6PD have been described (22). G6PD is an essential enzyme of the PPP, which is a metabolic pathway parallel to glycolysis that catalyzes the dehydrogenation of G6P to produce 6-phosphogluconate. 6-phosphogluconic acid undergoes a series of chemical reactions to produce 6-phosphofructose, which in turn can enter the glycolytic pathway or aerobic oxidation pathways. PPP has three important functions: i) PPP is the only means of using glucose to produce ribose 5-phosphate, and thus provides raw materials for nucleic acid synthesis *in vivo*. PPP can protect and stabilize DNA, which may make cancer cells more resistant to chemoradiotherapy damage (23-26). ii) PPP provides NADPH and stabilizes the antioxidant defense NADP/NADPH balance. NAPDH acts as an antioxidant and is used to detoxify high levels of reactive oxygen species (ROS) produced during rapid cell multiplication, thereby promoting cell survival (21,27). iii) Pentose enters glycolysis through the PPP (Fig. 1). In mammals, the PPP occurs solely in the cytoplasm, and is found to be most active in the human liver, mammary gland and adrenal cortex. NADPH and pentose supply are prerequisites for runaway growth and proliferation of cells, particularly in tumor cells (28). In addition to serving a role in cell proliferation and aging, G6PD may also be involved in the transmission of apoptotic signals (29). Another study found that highly glycosylated G6PD increases glucose uptake in the PPP and increases its activity. Therefore, blocking the glycosylation of G6PD may reduce the proliferation of cancer cells *in vitro* and impair tumor growth *in vivo* (30).

Being a functional pathway independent of glycolysis and oxidative phosphorylation, PPP also serves a crucial role in the liver (31), adipose tissue (32), gonads (33), bone marrow (34), red blood cells and other tissues (1). Since red blood cells do not contain mitochondria, the PPP is the only source of NADPH; therefore, protection from oxidative damage largely relies on G6PD (35). Tumor cell metabolism involves a number of metabolic pathways, including glucose transport, glycolysis, PPP, glutamine metabolism and the electron transport chain (36). Glycolysis refers to the process of transformation of glucose or glycogen into lactic acid and releasing energy through several intermediate steps in the absence of oxygen. Normally, cancer cells metabolize glucose, lactate, glutamine, pyruvic acid, acetate and aliphatic acids at markedly higher rates compared with normal cells (37). Cancer cells take advantage of conventional oxidative metabolism and glycolytic metabolism at the same time. However, even under conditions of sufficient oxygen, proliferation of cancer cells is marked by increased glycolytic metabolism (38). This phenomenon is termed the Warburg effect. The Warburg effect, first described by the German biochemist Otto Warburg, is a metabolic feature of aerobic glycolysis under conditions of sufficient glucose in tumors (5,39). Since the rate of ATP produced by the subsequent steps of glycolysis is 100 times faster compared with that produced by oxidative phosphorylation, as is seen in cancer, cells that rely on oxidative phosphorylation have an advantage since glucose concentrations are higher (40,41). Although it is somewhat slower to convert glucose to ATP compared with other routes, glucose is the most plentiful nutrient present in the blood, and is a metabolic substrate commonly used by tumor cells (39).

The metabolic biology of tumors is complex, and a growing body of evidence suggests that cancer cells have a unique metabolic program that allows for rapid cell proliferation (42). Previous studies have demonstrated that G6PD expression or enzymatic activity is increased in several types of tumors, including colorectal (43), myeloma (44), bladder (11), breast (45), gastrointestinal (46), esophageal (47) and prostate cancer (13) In addition, the instability of G6PD expression is also associated with the degree of malignancy of the tumor. There is increasing evidence that G6PD deficiency affects nucleated cells and cellular pathophysiology, which includes cell proliferation disorders, accelerating cell senescence, increasing susceptibility to viral infections and impairing embryonic development (48,49). The energetic and biosynthetic demands of rapidly proliferating cells, and the relatively balanced levels of ROS regulated by G6PD, may highlight a means of treatment for patients with cancer.

3. Abnormal status and function of G6PD in cancer cells

The nutrient demands and energy flow rates of tumor cells are often higher compared with those of normal cells. It was previously confirmed that regulation of tumor cell metabolism may affect the status of the cancer (50). Rapidly dividing cancer cells require three basic metabolic events: The formation of ATP, macromolecular synthesis and cell assembly, and an appropriate cellular redox environment (51). G6PD serves a key role in maintaining a normal redox potential in cells by reducing $NADP^+$ to NADPH in the PPP, the dysregulation of which results in insufficient antioxidant defense (52). Changes in the G6PD state are associated with numerous pathophysiological cellular alterations and diseases, including oxygen deficits (53), inflammation (54), infection (55), septicemia (56), diabetes (7), high blood pressure (57) and kidney diseases (14), amongst others. G6PD affects tumor development by regulating several metabolic pathways (14,54). The expression and activity of G6PD has been shown to be associated with the degree of malignancy of a variety of tumors. It has been demonstrated that blocking 80% of the G6PD activity in tumor cells significantly reduced cell proliferation, migration, invasion, and colony-formation; in addition, tumor cell apoptosis was increased (58). Using GEPIA (version 2019; gepia.cancer-pku.cn) combined with customizable functional analysis (such as tumor/normal tissue differences) to examine and mine data on multiple types of tumors in Genotype Tissue Expression (gtexportal.org/home/) and The Cancer Genome Atlas (portal.gdc.cancer.gov/), the clinical role of G6PD was further explored (Fig. 2A and B). Marked alterations of G6PD gene expression was observed in samples from 9 types of tumors [cholangio carcinoma, colon adenocarcinoma, acute myeloid leukemia (AML), HCC, rectal adenocarcinoma, pancreatic adenocarcinoma, cutaneous melanoma, stomach adenocarcinoma and testicular tumor] compared with normal tissues (Fig. 2A). Furthermore, overall survival analysis revealed that G6PD overexpression was associated with a poor prognosis in certain types of cancer, including mesothelioma, invasive breast carcinoma, HCC, AML and low-grade brain glioma

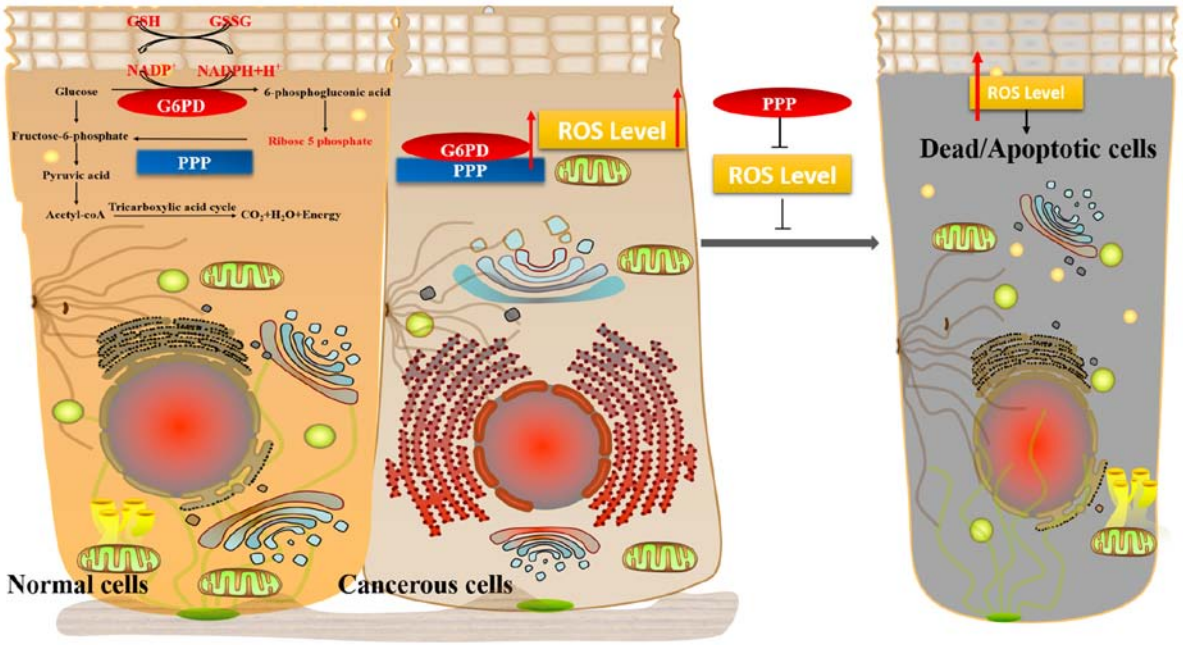

Figure 1. G6PD participates in the PPP in the cytoplasm. G6PD is a key enzyme of the PPP. Glucose metabolism via this pathway primarily produces NADPH and ribose 5-phosphate, which are raw materials for required biosynthesis of other components, and also serve an antioxidant role. The function of ROS in cells is dependent on its levels. Disruption of the ROS balance promotes tumorigenesis and cell death. G6PD, glucose-6-phosphate dehydrogenase; PPP, pentose phosphate pathway; ROS, reactive oxygen species.

(Fig. 2B). Thus, abnormal expression of GP6D is a common finding observed in tumorigenesis, and G6PD is potentially carcinogenic.

Studies on several types of cancer have demonstrated that overexpression of G6PD promotes tumorigenesis and development (summarized in Table I).

Liver cancer cells. In HCC, the phosphatase and tensin homologue (PTEN) signaling pathway is downregulated compared with normal tissues, and its ability to inhibit tumorigenesis is reduced (59). PTEN can inhibit the formation of dimers of the G6PD holoenzyme (60). In advanced stage liver cancer, the expression of G6PD is significantly increased, whereas expression of PTEN is decreased (60). Huh-sh-heterogeneous nuclear ribonucleoprotein (hnRNPK) is known to regulate G6PD-mRNA splicing. It has been demonstrated that T-cell leukemia 1 (Tcl1) affects cell resistance in HCC. Tcl1 directly interacts with hnRNPK *in vitro* to competitively inhibit the binding of hnRNPK and G6PD. Conversely, increasing evidence is indicating that PTEN disrupts the interaction between Tcl1 and hnRNPK by inactivating Tcl1 (59). In summary, PTEN induces the expression of G6PD through the Tcl1/hnRNPK/G6PD axis, and thus promotes hepatocarcinogenesis.

In HCC cell lines, it was found that the number of β-galactosidase-positive cells and expression of p21 (a classical aging marker) increased significantly following G6PD gene knockout, which indicated that the decrease of G6PD delayed the growth of tumor cells (60). The possible implications of this in the treatment of liver cancer is that G6PD inhibition may increase the sensitivity of liver cancer cells to chemotherapeutic drugs (61). Chronic hepatitis B virus (HBV) infection is considered to be involved in the pathogenesis of HCC. Therefore, the role of G6PD in HBV is also associated with its effect on liver cancer. It has been reported that G6PD may be associated with HBV replication, due to the fact that small interfering RNA-mediated silencing of G6PD reduced HBV production. In addition, when G6PD was silenced, the concentration of HBsAg and HBeAg secreted in the supernatant was also significantly reduced (62).

Kidney cancer. Renal cell carcinoma (RCC) is the most common type of renal malignancy, accounting for ~3% of all types of tumors (63,64). RCC is the most aggressive type of urinary tract tumor (63,65). Several studies have concluded that RCC can be viewed as a metabolic disease, the occurrence of which is affected by metabolic changes (66-68). G6PD is not only upregulated in all types of RCC specimens, but also exhibits increased activity in RCC cells (52). Elevated G6PD expression is associated with shorter overall survival. It has been confirmed that the median survival of the patients with high G6PD expression was significantly shorter compared with that of patients with low G6PD expression (69). G6PD overexpression promotes RCC cell proliferation, whereas G6PD silencing reduces the rate of RCC cell proliferation *in vitro* and inhibits xenograft tumor development *in vivo* (70). It was previously demonstrated that G6PD may promote tumorigenesis by increasing the rate of proliferation and enhancing antioxidant defense (71), whereas G6PD disorders render tumor cells more adaptable to the immune response and more aggressive (72). The pathways involving G6PD in the proliferation of renal cancer cells are as follows: i) G6PD stimulates ROS production through NADPH oxidase 4 (NOX4); ii) ROS accumulation increases phospho-(p-)signal transducer and activator of transcription 3 (STAT3); and iii) G6PD activates p-STAT3/STAT3 signaling and enhances cyclin D1 expression (73). Briefly,

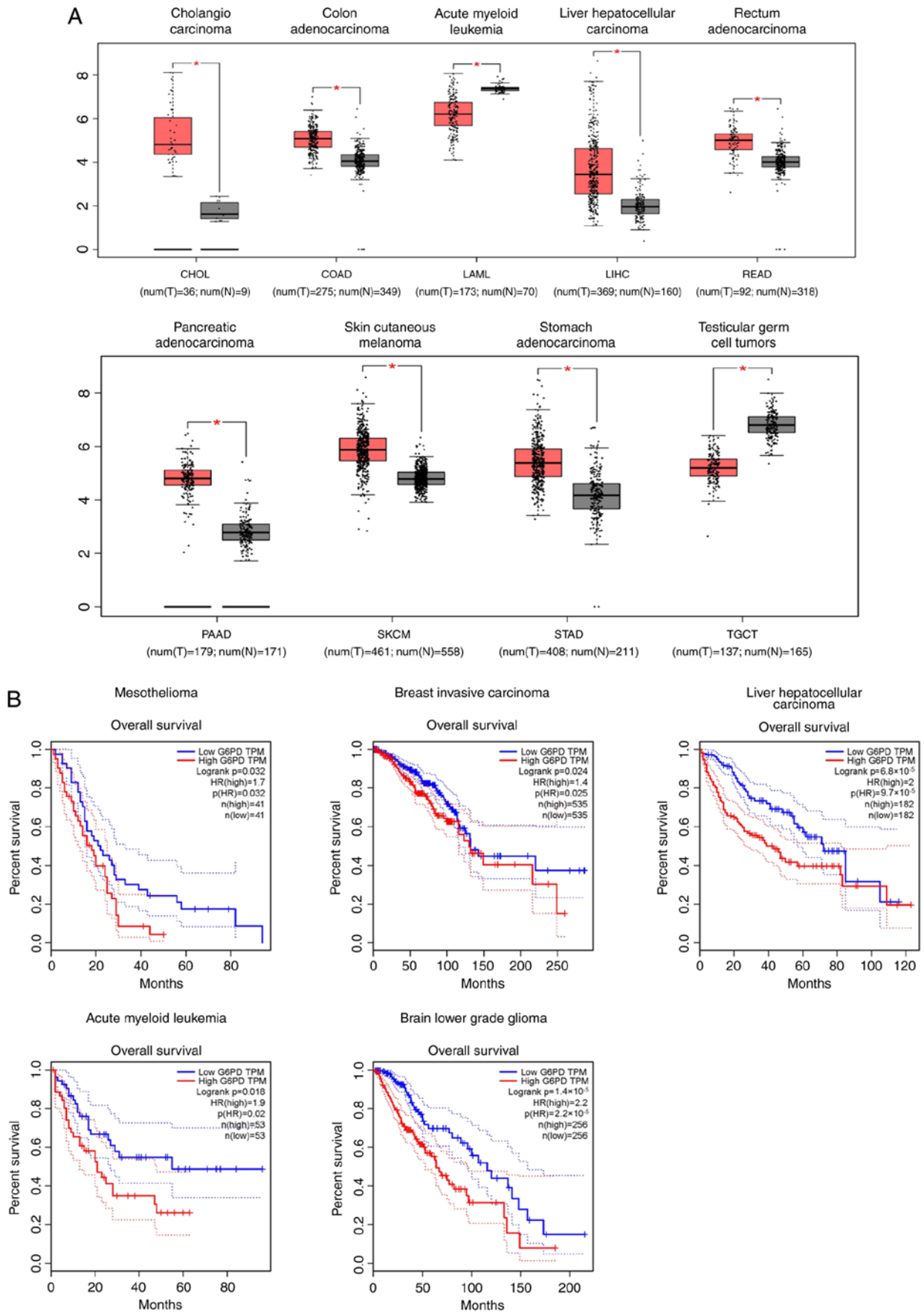

Figure 2. Association between the expression of G6PD and cancer. (A) Expression of G6PD and (B) association between G6PD and overall survival of patients with cancer. G6PD, glucose-6-phosphate dehydrogenase; CHOL, cholangiocarcinoma; BRCA, Breast invasive carcinoma; COAD, colon adenocarcinoma; HCC, hepatocellular carcinoma; AML, acute myeloid leukemia; LGG, low-grade glioma of the brain; MESO, mesothelioma; PAAD, pancreatic adenocarcinoma; READ, rectal adenocarcinoma; CM, cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; TPM, transcripts per million; T, tumor tissue; N, normal tissue; HR, hazard ratio. *P<0.05.

Table I. G6PD expression in different types of tumors.

First author, year	Cancer	Impact of G6PD upregulation	Mechanism	(Refs.)
Hong, 2013	Hepatocellular carcinoma	Liver cancer progression	PTEN affects G6PD active dimer formation via Tcl1/hnRNPK/G6PD	(60)
Zhang, 2017	Renal cell carcinoma	Upregulating the proliferation rate; increasing oxidative stress; altering the cell cycle	Activation of aG6PD/ROS/ p-STAT3/Cyclin D1 axis	(70)
Xu, 2016	Leukemia	Increasing proliferation and clonogenic activity; cell invasion	SIRT2 promotes NADPH production by deacetylating G6PD	(74)
Lu, 2018 Chow, 2010	Others	Lymph node metastasis; recurrence; lethal; metastasis	Summarized in Fig. 4	(61,65)

PTEN, phosphate and tension homology deleted on chromosome ten; Tcl1, T-cell leukemia/lymphoma protein; hnRNPK, hn heterogeneous nuclear ribonucleoprotein; ROS, reactive oxygen species; p-STAT3, phospho-signal transducer and activator of transcription 3; SIRT2, Sirtuin-2; G6PD, glucose-6-phosphate dehydrogenase.

G6PD can stimulate RCC tumorigenesis *in vivo* and *in vitro* by activating the G6PD/ROS/p-STAT3/cyclin D1 signaling pathway in RCC (70).

Leukemia. A number of studies on hematological malignancies have investigated the role of G6PD. It has been reported that NADPH produced by G6PD may be used for lipogenic reactions in leukemia cells (74). AML is a hematological malignancy and it is the most common type of adult acute leukemia (75), characterized by abnormal growth of myeloid progenitor cells and inhibition of cell differentiation (76). PPP, which is closely associated with glycolysis, is also frequently altered in AML. Glucose catabolism in leukemia cells may be mediated through the PPP rather than the glycolysis pathway, indicating that PPP may serve an important role in leukemia metabolism (74,77-80). It has been observed that AML cells rely on glucose metabolism for survival, and a high-flux of glucose is primarily maintained by PPP (81). The suppression of G6PD abolished glycolysis, weakened the effects of PPP and decreased the glucose utilization rate. As a result, AML cells exhibited arrested growth or death, and became more sensitive to chemotherapeutic drugs (82-84). *In vivo* mouse models have shown that enhanced glucose uptake accelerates the development of leukemia *in vivo* (75,85,86). Therefore, it may be concluded that G6PD is involved in tumor invasion by affecting other enzymes.

In terms of protein interactions, the deacetylation of G6PD by NAD-dependent deacetylase sirtuin 2 (SIRT2) can enhance the production of NADPH and promote the proliferation of leukemia cells (74). It is hypothesized that the binding of G6PD and SIRT2 is disrupted in heat shock protein 27 (HSPB1-L) knockout cells (87). When HSPB1 expression increases, it can promote the binding of SIRT2 and G6PD, in-turn affecting tumor cell proliferation. By activating the phosphoinositide 3-kinase/Akt/mammalian target of rapamycin signaling pathway, the activity of G6PD is regulated by sterol-regulatory-element-binding protein 1c, which ultimately promotes tumor cell transformation (28).

Other type of cancer. The role of G6PD in several other types of cancer are summarized below. In gastric cancer, the expression levels of G6PD protein was found to be associated with the Tumor-Node-Metastasis stage. The rate of G6PD protein expression in gastric cancer tissues with positive lymph node metastasis was also higher compared with that in lymph node-negative patients (46,88). Colorectal cancer (CRC) is the third most commonly diagnosed type of cancer in Western countries. Moreover, the morbidity rates of CRC have rapidly increased in China over the past two decades (61,89,90). It has been reported that G6PD expression is upregulated in CRC cells and patient specimens. Breast cancer has the highest incidence amongst all types of cancer in women, and remains one of the leading causes of cancer-related death (91). Upregulated expression of G6PD is considered a predictor of a high risk of recurrence and metastasis in patients with breast cancer (92). In prostate cancer, G6PD has been used as a biomarker of diagnosis and prognosis for 30 years (93). G6PD activity in patients with prostate cancer is 4-fold higher compared with that in patients with benign prostatic hyperplasia (93,94). Previous studies have shown that the G6PD levels increase via the PPP in prostate cancer (95-97). In glioma, the activity and content of G6PD affects the efficacy of chemotherapy and radiotherapy (52,98). When human melanoma was investigated using a G6PD knockdown xenograft mouse model, it was observed that G6PD expression was closely associated with tumor formation, size and weight (99). In bladder cancer, knockdown of G6PD inhibits tumor cell proliferation and colony formation (11).

Therefore, G6PD expression is frequently found to be elevated in several different types of cancer, and its internal mechanisms of action have become the focus of increasing attention.

4. Role of G6PD in cancer and the related networks

The mechanisms through which G6PD regulates tumor development are yet to be fully elucidated. It is well established

that G6PD levels are aberrantly elevated in several different types of cancer, and promotes the proliferation of cancer cells through production of ribose-5-phosphate and NADPH as discussed above. The most significant function of NADPH is to form two GSH molecules by reducing one GSH disulfide molecule (100). In turn, GSH may be used to defend against excessive accumulation of ROS (74). ROS, which are produced at low levels by the electron transport chain as part of physiological cellular metabolism, serve a physiologically important role in the regulation of cell signaling, proliferation and differentiation. When ROS production increases above a certain cell-dependent threshold, it may damage cellular components, resulting in cell death (101). Increased generation of ROS in metabolically active cells requires an appropriate level of antioxidants, such as the reduced form of GSH, produced by the PPP (102). It has been shown that ROS can contribute to tumorigenesis by activating various signaling pathways that control cell growth and proliferation (103). However, excess ROS also induces apoptosis (102). If ROS production is higher than the antioxidant defense capacity of the cell, it may lead to oxidative stress, thereby causing cell death. Furthermore, as an organisms ages, the capacity to repair ROS-induced injury becomes gradually weaker and the accumulation of ROS *in vivo* results in terminal damage (100). That is, when the expression or activity of G6PD gradually decreases, the antioxidant ability of the PPP is weakened and the levels of ROS progressively increases. G6PD participates in the PPP to promote the production of GSH. As a classic antioxidant, GSH can defend against excessive production of ROS, thereby maintaining cells, particularly tumor cells, in a proliferating state (Fig. 1). To resist oxidative stress, cancer cells have enhanced antioxidant programs (50). Thus, a key molecule produced as a result of altered cancer metabolism is NADPH, which is an antioxidant and forms part of the defense against ROS (50). There are two primary forms of NADPH production: i) NADH and $NADP^+$ generate NADPH by catalysis of mitochondrial transhydrogenase and ii) $NADP^+$ produces NADPH by catalysis of a variety of $NADP^+$-dependent enzymes (104). Currently, increasing evidence is suggesting that NAD (NAD^+ or NADH) and NADP ($NADP^+$ or NADPH) can influence various biological processes, including energy metabolism, mitochondrial functions, calcium homeostasis, antioxidation/generation of oxidative stress, gene expression, immunological functions, aging and cell death (104-107). In fact, the sources of NADPH generation may determine the different biological effects of NADPH: The NADPH catalyzed by the mitochondrial enzymes primarily contribute to antioxidation and biosynthesis, whereas the NADPH catalyzed by the cytosolic enzymes contributes to NADPH oxidase-dependent ROS generation when NADPH oxidase (NOX) is activated (104). The NOX family consists of seven enzymatic isoforms, and is an enzyme complex with the unique function of producing superoxide anions and ROS through consumption of NADPH (108). The process of NOX-mediated production of ROS is primarily through one-electron reduction of oxygen to superoxide depending on NADPH (109). NADPH is produced by glucose, once inside the cells as an intermediate of glycolysis, to generate glucose 6-phosphate (G6P). G6P continues to pass through the glycolytic pathway or is split with a hexose-phosphate shunt to produce NADPH (108). In tumor cells, ROS promotes cell proliferation via redox reactions. ROS modulates several signaling pathways associated with cellular transformation, inflammation, tumor survival, proliferation, invasion, angiogenesis and metastasis of cancer (110). For maintaining cell proliferation, the levels of ROS in cancer cells is elevated compared with normal cells (105,106,111,112). Excessive accumulation of ROS may lead to cell apoptosis or even death; however, tumor cells may maintain proliferation without being affected by ROS, primarily due to the ability of anti-oxidative stress with the participation of NADPH (50,74,101,102). In the PPP pathway, NADPH maintains the reduced state of GSH, thereby inducing resistance to ROS (102). This process is involved in the anti-ROS effects of NADPH (102). Therefore, it may be concluded that a moderate increase in G6PD activity is beneficial for avoiding the harmful effects of ROS. Of note, G6PD activity and its expression are increased by oncogenes, such as KRAS (113), and suppressed by tumor suppressors, such as P53 or PTEN (60,114). Below, a summary of the studies investigating the underlying mechanisms, with a focus on proliferation, cell cycle progression, apoptosis, invasion and migration is provided (Fig. 3).

G6PD promotes epithelial-mesenchymal transition (EMT) by activating the STAT3 pathway. and EMT serves a key role in enhancing tumor cell invasion and metastasis (61). G6PD is important for several cellular processes and enzymes using NADPH, including enzymes in the antioxidant system (21), including nitric oxide synthase and superoxide dismutase, both of which are NADPH-dependent enzymes (115) and can promote EMT and invasion of pancreatic cancer cells (116).

Regarding cell apoptosis, the expression levels of Bcl-2 and Bcl-xL, which are both inhibitors of apoptosis, were found to be reduced in cells with low G6PD expression levels. However, the expression of Fas was increased, and Fas can bind to the cytokines of the death receptor TNFRSF6/Fas and induce apoptosis caused by T-cell-mediated cytotoxicity. Athanogene 3 (BAG3) protein directly binds to G6PD to exert a tumor suppressor-like function in HCCs (117). The Bcl-2 associated BAG3 protein is involved in several cellular functions, including cell cycle, autophagy, cell growth and pathogen replication (118,119). Through Bcl-2, BAG3 suppresses dimerization and activity of G6PD. It is hypothesized that G6PD may affect apoptosis by interacting with apoptosis-related factors (71,99).

Although there are no definitive conclusions on the role of G6PD in regulating the cell cycle, the results of other studies in this area are summarized in the present review. It has been established that in cells lacking G6PD, downregulation of the cell cycle proteins cyclin D1 and E, and S100a4 is observed (99). The expression of cyclin D1 is decreased following G6PD knockout (120), and this affected cell growth and division via regulation of the G1/S transition (63,121). Additionally, G6PD overexpression can activate the STAT3 pathway, primarily through increasing the levels of p-STAT3. When the expression of G6PD is low, protein tyrosine kinase (c-SRC) and protein tyrosine phosphatase (SHP2) are suppressed. As c-SRC and SHP2 can regulate the

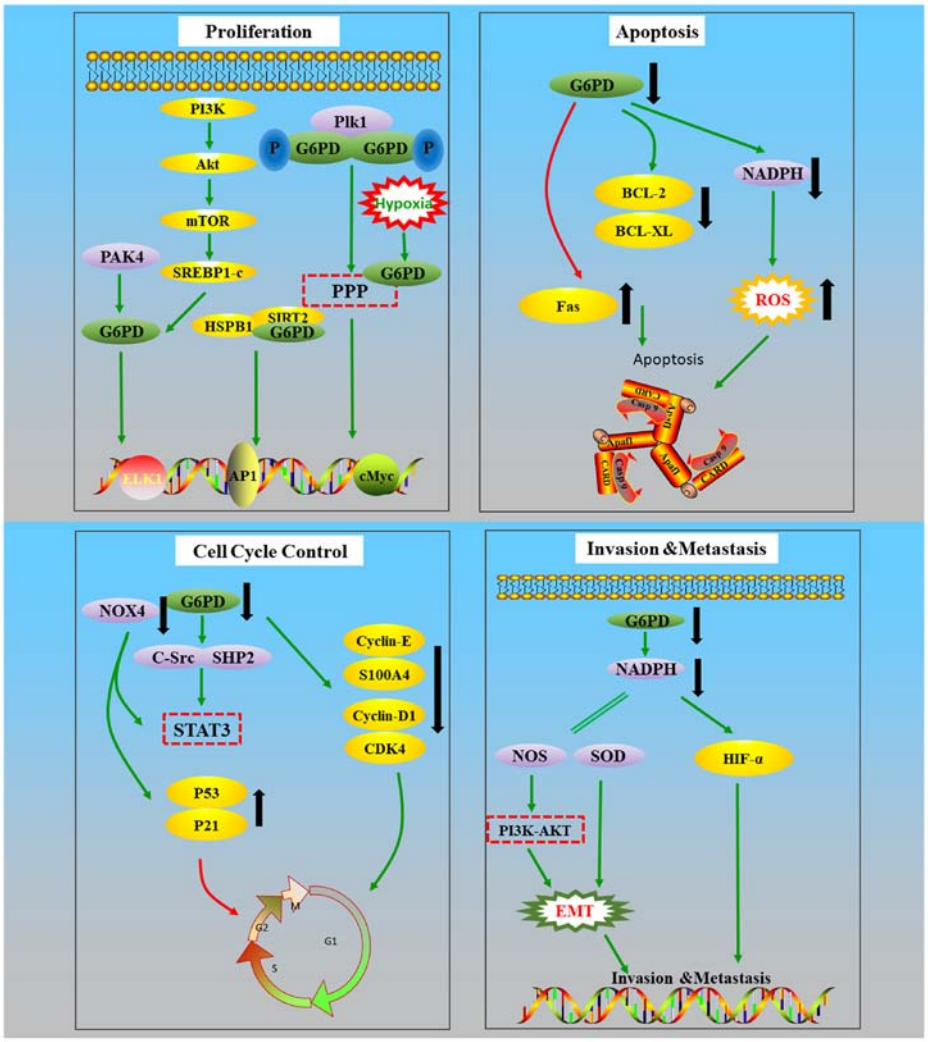

Figure 3. Schematic of the regulatory mechanisms of the involvement of G6PD in cancer, which primarily occurs via four aspects: Cell proliferation, apoptosis, cell cycle progression, and invasion and migration. G6PD, glucose-6-phosphate dehydrogenase; PPP, pentose phosphate pathway; ROS, reactive oxygen species; EMT, epithelial-mesenchymal transition; mTOR, mammalian target of rapamycin; CARD, Caspase recruitment domain; Casp 9, Caspase 9; SREBP1c, sterol-regulatory-element-binding protein 1c; SIRT2, sirtuin 2; HSP-B1, heat shock protein 27; NOX4, NADPH oxidase 4; c-Src, protein tyrosine kinase; SOD, superoxide dismutase; NOS, nitric oxide synthase; STAT3, signal transducer and activator of transcription 3; p, phospho; HIF-1α, hypoxia-inducible factor-1α; SHP2, protein tyrosine phosphatase; PI3K, phosphoinositide 3-kinase.

DNA-binding activity of STAT3, G6PD serves an important indirect role in DNA-binding via the above mentioned pathways, thus affecting cell cycle progression and suppressing cell proliferation (120). After silencing G6PD and NOX4, the expression levels of p53 and p21 were upregulated and the cell cycle was arrested in the G1/S phase. At the same time, the protein levels of cyclin D1 and cyclin-dependent kinase 4 were decreased. Upregulation of p53 may be achieved by downregulating PAK4, resulting in S phase arrest of the cell cycle (122). PAK4 can also directly interact with G6PD, and then enhance the activity of G6PD to promote glucose intake and the production of NADPH, thereby promoting tumor growth (123). These results indicate that the cell cycle is also affected by this pathway. Polo-like kinase 1 (Plk1), a serine/threonine kinase, regulates cell mitosis (124). G6PD is activated when Plk1 binds to G6PD. Moreover, G6PD-mediated PPP may be involved in the regulation of the cell cycle by Plk1 (124). By contrast, p53 inhibits the proliferation of tumor cells by repressing the formation of G6PD dimers (70). The mechanism by which G6PD affects the cell cycle is debated. In G6PD-deficient cells, there was a noticeable delay in cell proliferation, which was found to resemble cell senescence (29).

In an anoxic environment, G6PD also serves a regulatory role in the survival of tumor cells. Abnormal acetylation was found to be associated with the growth and invasion of cancer cells *in vitro* and *in vivo* (125,126). Under hypoxic conditions, the glycosylation of G6PD is activated, thus activating the PPP (30). Hypoxia-inducible factor-1α (HIF-1α) is present in abundant quantities in a variety of human tumor cells, and serves an important role in promoting tumor angiogenesis, accelerating tumor cell proliferation, and promoting invasion and metastasis (127). It has been suggested that the G6PD gene may impair the stability and expression of HIF-1α by downregulating the levels of NADPH, which may in-turn affect the hypoxia response of tumor cells (127). However, the detailed mechanisms by which G6PD affects tumor cells remain unknown.

Investigation of the mechanism by which G6PD promotes tumorigenesis and tumor development may improve our understanding of the association between G6PD and cancer, and may also provide theoretical guidance for targeted therapy.

5. G6PD and cancer treatment

Successful treatment, recurrence and resistance of cancer are commonly encountered problems. The results of traditional methods of cancer treatment (including surgical chemotherapy, radiotherapy, use of traditional Chinese medicines and biological therapy) are not optimal. Although first-line treatment with cytotoxic chemotherapy has achieved a high rate of remission, the majority of the patients eventually succumb to the disease. In order to increase the cure rates, improve the prognosis, and to minimize the suffering of the patients, more effective and less toxic cancer treatments are urgently required. Molecular targeted cancer treatment may hold promise as a therapeutic approach. The expression of G6PD may represent a potential treatment option. As the effect of G6PD activity on the cell cycle has been demonstrated, several pathways that involve G6PD may be targeted to improve the efficacy of treatment. The interactions between G6PD and multiple signaling pathways directly or indirectly, affects cell cycle progression of tumor cells, eventually altering cell proliferation or apoptosis. Ongoing studies in different types of cancer, and investigation of G6PD specificity may highlight potential avenues to overcome these problems. The PPP may also affect the adaptation of tumor cells to the changes in the microenvironment, and enable them to survive longer compared with normal cells (128,129). Although the detailed mechanisms of the functional mechanisms of G6PD are not fully understood, further investigations should address the following possibilities: i) The defense of tumor cells against oxidative stress may be weakened by inhibiting G6PD to interrupt the energy supply, leading to increased sensitivity of tumor cells to oxidants. ii) Based on the principle of metabolic reprogramming, by adjusting the activity of G6PD to affect the role of PPP in biosynthesis, the resistance of tumor cells to drugs may be overcome. iii) Alternatively, certain types of cancer cells may adapt to high levels of carcinogenic signals by destroying their aging or apoptotic induction circuits. G6PD may be involved in this process, or it may enable tumor cells to escape certain events that have a negative effect on cancer cell proliferation.

The metabolic reprogramming alters the tumor microenvironment affecting cancer cells and other immune cells, such as macrophages, T lymphocytes and myeloid-derived suppressor cells (5,130). Targeting metabolic transformation or metabolism-regulated signaling pathways in tumor development may be a potential anticancer treatment strategy, alone or in combination with immunotherapy. At present, as one of the basic characteristics of tumor cells, metabolic programming has been recognized as a new cancer hallmark (130). In order to meet the needs of rapid proliferation for energy and to counteract the increased oxidative stress, tumor cells are reprogrammed, and this includes changes to their glucose, fat and other metabolic pathways. Ribose-5-phosphate and NADPH are important products catalyzed by G6PD. The former is the raw material of nucleic acid synthesis (108), which is the basis of cell division, proliferation and maturation, whereas the latter is involved in fatty acid, nucleic acid and ROS metabolism, amongst other functions, acting as a hydrogen donor in all these processes (104). In this context, G6PD serves an important role in promoting biosynthesis, and an increase of G6PD activity further promotes the activity of PPP. As a result, a large quantity of glucose is consumed, which is involved in numerous biosynthetic processes and this produces a large set of reductants (23-26). Thus, it is hypothesized that metabolic reprogramming by PPP may be of value as a biomarker for detection of cancer and as a target for the development of novel anticancer treatments (36).

G6PD inhibitors have been developed, and 1-bromoacety l-3,3-dinitroazetidine (RRx-001) is one of these. RRx-001 is both a G6PD inhibitor and a multifunctional anticancer drug that has been tested in phase III clinical trials (131). RRx-001 has been evaluated in ~300 patients in 9 clinical trials (131), and the results were promising, with no associated hematological toxicities reported. Based on the proven antitumor activity and favorable toxicity profile in the phase II clinical trial, RRx-001 has been approved by the FDA and EMA, and may be used in phase III multi-center studies in subjects with relapsed/refractory solid tumors (131,132). However, *in vitro* experiments have shown that high concentrations (7-10 μM) of RRx-001 reduce the viability of peripheral mononuclear blood cells by 30-70%, indicating that normal cells are not entirely refractory to RRx-001 (133). Polydatin is another known G6PD inhibitor, which may cause ROS accumulation and enhanced endoplasmic reticulum stress by inhibiting G6PD (134). Experiments have shown that polydatin is non-toxic to animals at a dose of 200 mg/kg (134). Phase II clinical trials demonstrated that it is also well tolerated in humans at a dose of 40 mg twice a day for 90 days. Therefore, polydatin may be used with confidence in clinical treatment (134). Thus, it appears that the toxicity of G6PD inhibitors to normal cells is limited, but not completely absent. However, G6PD is still hypothesized to serve as a promising target for anti-cancer treatments.

6. Conclusion

In summary, G6PD may be characterized as a participant rather than a bystander in the process of tumorigenesis, and downregulation of G6PD may enhance the sensitivity of certain types of tumors to chemotherapeutic drugs. Therefore, G6PD may serve as an important target for cancer therapy and for overcoming resistance to chemotherapy.

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Availability of data and materials

The data used for the analyses described in this manuscript were obtained from the GTEx (version 2019; gepia.cancer-pku.cn) and The Cancer Genome Atlas (portal.gdc.cancer.gov/).

Authors' contributions

YY and CG conceived the subject of the review. RL and WW wrote the manuscript, analyzed the data, and plotted the graphs. CG and YY edited the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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