

Data mining and pathway analysis of glucose-6-phosphate dehydrogenase with natural language processing

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Abstract. Human glucose-6-phosphate dehydrogenase (G6PD) is a crucial enzyme in the pentose phosphate pathway, and serves an important role in biosynthesis and the redox balance. G6PD deficiency is a major cause of neonatal jaundice and acute hemolytic anemia, and recently, G6PD has been associated with diseases including inflammation and cancer. The aim of the present study was to conduct a search of the National Center for Biotechnology Information PubMed library for articles discussing G6PD. Genes that were identified to be associated with G6PD were recorded, and the frequency at which each gene appeared was calculated. Gene ontology (GO), pathway and network analyses were then performed. A total of 98 G6PD-associated genes and 33 microRNAs (miRNAs) that potentially regulate G6PD were identified. The 98 G6PD-associated genes were then sub-classified into three functional groups by GO analysis, followed by analysis of function, pathway, network, and disease association. Out of the 47 signaling pathways identified, seven were significantly correlated with G6PD-associated genes. At least two out of four independent programs identified the 33 miRNAs that were predicted to target G6PD. miR-1207-5P, miR-1 and miR-125a-5p were predicted by all four software programs to target G6PD. The results of the present study revealed that dysregulation of G6PD was associated with cancer, autoimmune diseases, and oxidative stress-induced disorders. These results revealed the potential roles of G6PD-regulated signaling and metabolic pathways in the etiology of these diseases.

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) is a critical enzyme in the pentose phosphate pathway (PPP). G6PD serves an important role in nucleic acid synthesis via generation of ribose 5-phosphate, and in the cellular response to oxidative stress through the generation of reduced nicotinamide adenine-dinucleotide phosphate (NADPH) (1,2). G6PD deficiency was the first prevalent enzyme deficiency characterized by the World Health Organization in 1984 (3). It is an X-linked, hereditary genetic defect involving mutations in the G6PD gene, which lead to specific amino acid substitutions that alter enzymatic function (4). Globally, >400 million people suffer from G6PD deficiency (5). The most frequent clinical manifestations of G6PD deficiency are neonatal jaundice and acute hemolytic anemia, which are initiated by exogenous oxidative agents, including drugs, infection or ingestion of fava beans (4,6).

As G6PD activity is essential for preventing reactive oxygen species (ROS)/serum starvation-mediated cell death, dysregulation of G6PD activity may lead to the development of disease (7). G6PD deficiency predisposes human foreskin fibroblasts to retarded growth and accelerated cellular senescence (8). Knocking out G6PD is embryonically lethal, and leads to cellular sensitivity to H₂O₂ and additional oxidative agents (9,10). By contrast, overexpression of G6PD in NIH 3T3 cells leads to anchorage-independent growth (11). Decreased G6PD activity may predispose humans to type 1 and type 2 diabetes (12,13). In addition, G6PD has been associated with hypertension (14).

Increased G6PD activity has been reported in various neoplasms, including hepatocellular cancer, leukemia, colorectal cancer, breast tumors, renal cell carcinomas, and gastrointestinal cancers (15-20). A previous study demonstrated that silencing of G6PD in human A375 malignant melanoma cells downregulated cyclin D1, cyclin E, p53, S100 calcium-binding protein A4 and the apoptosis factor Fas, and upregulated the apoptosis-inhibiting factors B-cell lymphoma (Bcl) 2 and Bcl-extra large (21). G6PD regulates apoptosis and the proliferation of A375 cells, potentially via the signal transducer and activator of transcription (STAT) 3/5 signaling pathway (22).

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MicroRNAs (miRNAs) are a class of single-stranded molecules, 20 to 22 nucleotides in length, which constitute approximately 0.01% of total RNA. By binding to specific sites within the 3'-untranslated region, miRNAs inhibit the translation of multiple mRNA targets (22). miRNAs have been reported to serve critical roles in embryonic development, cell proliferation, the cell cycle, apoptosis, neovascularization, inflammation, tumorigenesis and various additional diseases (23-33). Therefore, determining whether any miRNAs regulate G6PD may provide an insight into G6PD-associated biological processes and diseases.

miRNAs function to promote, as well as suppress tumor development. For instance, overexpression of the oncogenic miRNA-155 in transgenic mice leads to B cell proliferation, which develops into B cell leukemia and aggressive lymphoma (23). By contrast, tumor suppressive miRNAs prevent tumors from developing by negatively regulating oncogenes and/or genes controlling proliferation or apoptosis. The expression of *let-7*, which was one of the first miRNAs discovered, is downregulated in lung cancer (34). Overexpression of *let-7* is capable of inhibiting cancer cell growth *in vitro* and *in vivo*, while down-regulation of *let-7* impairs the survival of various types of tumor (25,26,35). In addition, the expression levels of several miRNAs have been reported to correlate with tumor metastasis. For instance, the expression level miRNA-422a is negatively correlated with metastasis of hepatocellular carcinoma (HCC), and miRNA-422a is significantly downregulated in HCC (33). Overexpression of miR-422a in HCC tumor cells significantly inhibits tumor growth and liver metastasis in xenograft tumor models (33).

In addition to cancer, miRNAs are involved in various additional biological processes and diseases. An miRNA cluster at chromosome 14q32 participates in neovascularization, and inhibition of 14q32 miRNAs, including miR-329, miR-487b, miR-494 and miR-495 promotes neovascularization and recovery of blood flow following ischemia (30,33). In addition, a number of miRNAs influence energy metabolism. By repressing cardiac peroxisome proliferator-activated receptor- Δ , miR-199a facilitates a metabolic shift from a predominant reliance on fatty acid utilization toward an increased reliance on glucose metabolism, which leads to lethal heart failure (36). Inhibition of miRNA binding improved cardiac function and restored mitochondrial fatty acid oxidation in animal models, thus highlighting a novel potential therapy for heart failure (36).

To the best of the author's knowledge no systematic studies investigating the correlation between G6PD, G6PD-associated genes, and miRNAs regulating G6PD have been conducted to date. Therefore, 2,253 articles retrieved from the National Center for Biotechnology Information (NCBI) PubMed library were used to investigate the networks of G6PD-associated genes in the present study. A total of 98 genes and 7 signaling pathways were demonstrated to be significantly correlated with G6PD. In addition, 33 miRNAs that may modulate the expression or activity of G6PD were identified.

Materials and methods

Data selection, extraction and filtering for the natural language processing (NLP) analysis of human G6PD. The PubMed library was first searched for articles regarding G6PD

deficiency that were published between January 1980 and June 2014, using the following keywords: 'Glucosephosphate Dehydrogenase Deficiency'[Mesh] AND '1980/01/01'[Date-Publication]: '2014/06/01'[Date-Publication]. All abstracts were downloaded as HTML text without images and converted into XML documents. The genes and proteins reported were compiled into a data pool, and gene mention recognition was performed using A Biomedical Named Entity Recognizer version 1.5 tool (<http://pages.cs.wisc.edu/~bsettles/abner/>) (37), and conjunction resolution was conducted to obtain specialized descriptions of the extracted genes. For instance, 'CASP 3/7 gene' was divided into the CASP 3 genes and CASP 7 genes. In order to normalize the variety of gene aliases identified, all genes were unified according to the Entrez gene official symbols (<http://www.ncbi.nlm.nih.gov/gene>) (38-42). The process of NLP is illustrated in Fig. 1.

Statistical analysis of selected data. For all publications retrieved from the PubMed database (N) the number of genes mentioned in the PubMed database for G6PD (m) and associated genes (n) were recorded. The frequency at which each gene appeared was calculated. The rate at which each gene was mentioned was assumed to be associated with the likelihood that it was correlated with G6PD, and the number of articles in which a given gene was associated with G6PD (k) was recorded. By employing the hypergeometric distribution, the probability of a frequency greater than k under completely random conditions was calculated using the following formula:

$$p = 1 - \sum_{i=0}^{k-1} p(i | n, m, N)$$

where,

$$p(i | n, m, N) = \frac{n!(N-n)!m!(N-m)!}{(n-i)!i!(n-m)!(N-n-m+i)!N!}$$

The G6PD-gene associations where the p-value was equal to <0.05, were subsequently summarized and subjected to a linked database (<http://www.r-project.org/>; <http://www.bioconductor.org/packages/2.4/bioc/html/KEGGSOAP.html>; <http://www.genmapp.org/>; <http://mips.helmholtz-muenchen.de/proj/ppi>) for further analysis.

Gene ontology (GO) analysis. Enrichment of gene sets was analyzed using the GOSTats software package (<http://www.bioconductor.org/packages/release/bioc/html/GOstats.html>) (39). GO analysis was implemented through the GSEABase package version 1.4.0 from The R Project for Statistical Computing (<http://www.r-project.org/>) (43). Genes were classified according to biological process, cellular component and molecular function.

Pathway and gene network analysis. The G6PD-associated genes were mapped to the Kyoto Encyclopedia of Genes and Genomes pathway database (KEGG; <http://www.kegg.jp/>) using the GenMAPP version C3 (<http://www.genmapp.org/>), and the enrichment p-value was calculated for each pathway (44,45). Based on the protein interactions, regulated genes and protein modifications listed in the KEGG

Table I. Top ten significant glucose-6-phosphate dehydrogenase-associated genes.

Entrez gene ID	Official gene symbol	Gene definition	PubMed count	P-value
2936	GSR	Glutathione reductase	44	$<1.00 \times 10^{-3}$
3630	INS	Insulin	40	$<1.00 \times 10^{-3}$
847	CAT	Catalase	38	4.80×10^{-9}
2908	NR3C1	Nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)	23	$<1.00 \times 10^{-3}$
6647	SOD1	Superoxide dismutase 1, soluble	17	$<1.00 \times 10^{-3}$
3251	HPRT1	Hypoxanthine phosphoribosyltransferase 1	7	$<1.00 \times 10^{-3}$
2157	F8	Coagulation factor VIII, procoagulant component	6	3.96×10^{-5}
100	ADA	Adenosine deaminase	3	1.21×10^{-3}
10327	AKR1A1	Aldo-keto reductase family 1, member A1 (aldehyde reductase)	3	1.75×10^{-5}
3439	IFNA1	Interferon, α 1	3	2.15×10^{-3}

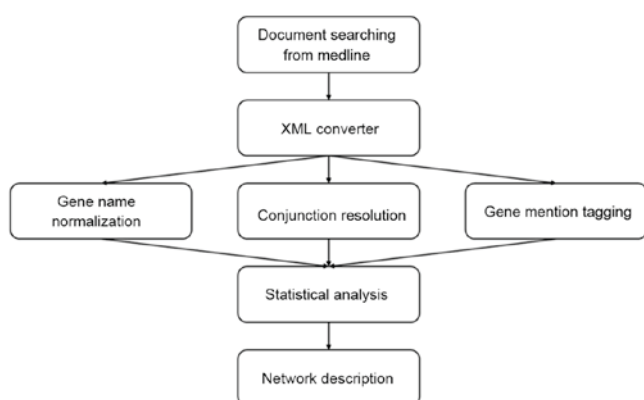


Figure 1. A flow chart of the natural language processing strategy employed in the present study. A total of 2,253 articles were obtained by querying the National Center for Biotechnology Information PubMed library with: 'Glucosephosphate Dehydrogenase Deficiency'[Mesh] AND '1980/01/01'[Date-Publication]: '2014/06/01'[Date-Publication]. All the abstracts were downloaded as HTML text and converted into XML documents. Following ABNER tagging, all of the genes that were mentioned were normalized according to the Entrez database. ABNER, a biomedical named entity recognizer.

database, the G6PD-associated genes were integrated into the following three different interaction associations: Enzyme-enzyme interactions, representing two enzymes catalyzing successive reaction steps; protein-protein interactions, representing binding and modification; transcription factor-target gene product interactions, representing gene expression interactions. In addition, the KEGG database was used to investigate the differentially expressed genes associated with metabolism and disease signaling pathways. Briefly, the results of the signaling pathway analysis was downloaded from the KEGG database, and the interactions between genes were analyzed using the KEGGSOAP package version 2.4 (<http://www.bioconductor.org/packages/2.4/bioc/html/KEGGSOAP.html>) derived from The R Project for Statistical Computing (<http://www.r-project.org/>) (46,47). Protein-protein interaction data was downloaded from the MIPS Mammalian Protein-Protein

Interaction Database (<http://mips.helmholtz-muenchen.de/proj/ppi/>) (48). The frequency of the co-citation of each gene was calculated employing co-citation matrices to identify a specific gene term and its variants included within an abstract. Statistical analysis was performed using the same methods described for the NLP analysis of G6PD, and the subsequent network was visualized using the open-source Medusa software program version 3.0 (<https://sites.google.com/site/medusa3visualization>) (49). Genes were classified according to associated signaling pathways. Genes involved in various signaling pathways were assigned to the single pathway with the smallest enrichment P-value. Following integration of the PubMed articles, the collection of protein-protein interaction and conjunction resolution information, and additional results from the Search Tool for the Retrieval of Interacting Genes/Proteins tool version 2.0 (<http://string-db.org/>) (50), a network of G6PD-associated genes was established.

Prediction of miRNAs targeting G6PD. The miRNAs targeting G6PD were identified using four independent software programs: TargetScan 6.2 (<http://www.targetscan.org/>) (51); miRanda (version 5.0; <http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/>); starBase (version, 2.0; <http://starbase.sysu.edu.cn/browseIntersect-TargetSite.php>) (52) and PicTar (http://pictar.mdc-berlin.de/cgi-bin/PicTar_vertbrate.cgi?species=vertebrate).

Results

Natural language processing analysis of G6PD. A search of the PubMed library for articles discussing G6PD deficiency that were published between January 1980 and June 2014, identified 2,253 primary G6PD-associated studies (Fig. 1). Each gene mentioned in these articles was recorded, and the top five most cited genes were glutathione-disulfide reductase (GSR), insulin (INS), catalase (CAT), superoxide dismutase (SOD1) and adenosine deaminase (ADA; Table I). Each gene mentioned in these articles was categorized using

Table II. Gene ontology analysis of significant genes obtained from the natural language processing analysis.

A, Cellular component			
Term	Genes	Count	P-value
Mitochondrion	CAT, NOS1, HK1, MPO, BCL2, SOD1, GCK, CYB5R3, ATP5A1, GSR	10	1.50x10 ⁻²
Endoplasmic reticulum/Golgi	NOX4, UGT1A1, SGK1, BCL2, PRKG1, PTGS2, HSPA5, EPHX1, ATP7B, POR, CYB5R3, NOS3	12	1.42x10 ⁻²
Additional cytoplasmic organelles	CAT, TNF, MPO, GLA, ATP6AP1, XDH	6	2.13x10 ⁻²
Cytosol	CCND1, NR3C1, HK1, BCL2, HSP90AA1, NADK, MDH1	7	1.32x10 ^{-3a}
Plasma membrane	EGF, L1CAM, SLC6A8, NOX4, VASP, NOS1, ATF4, TNF, UGT1A1, INSR, FPR1, BCL2, EGFR	21	1.00x10 ⁻²
Non-structural extracellular	FST, EGF, IL1B, CSF3, F8, TNF, PTH, IFNA1, LPL, LTF, IL6, SOD1	17	8.03x10 ^{-4a}
Additional cellular components	CCND1, L1CAM, IL1B, AR, NR3C1, SORD, VASP, NOS1, ATF4, TNF, NASP, ESR1	43	7.67x10 ^{-3a}
B, Molecular function			
Term	Genes	Count	P-value
Transcription regulatory activity	AR, NR3C1, ATF4, ESR1, CREM, BCL2, STAT3, PGR, CREB1, SMARCA5, CDKN2A	11	2.15x10 ⁻²
Kinase activity	CCND1, INSR, HK1, PGK1, SGK1, PDGFRB, EGFR, PRKG1, GCK, NADK, CDKN2A, IKBKG	12	9.20x10 ^{-4a}
Additional molecular functions	TKT, CCND1, FST, EGF, L1CAM, SLC6A8, AR, CAT, GSS, NR3C1, SORD, NOX4	75	2.08x10 ^{-3a}
C, Biological process			
Term	Genes	Count	P-value
Cell cycle and proliferation	CCND1, FST, EGF, IL1B, AR, CAT, NOX4, TNF, NASP, ESR1, PTEN, ADA	23	4.13x10 ^{-8a}
Transport	IL1B, SLC6A8, NOS1, TNF, NASP, SGK1, ADA, BCL2, SLC2A1, CREB1, NOX1, LTF	22	1.75x10 ⁻²
Stress response	CCND1, IL1B, CAT, F8, TNF, MPO, IFNA1, SGK1, ADA, BCL2, STAT3, HSPB1	19	3.75x10 ⁻⁶
Developmental processes	CCND1, FST, EGF, L1CAM, IL1B, AR, NR3C1, NOX4, CSF3, VASP, TNF, INSR	41	1.21x10 ^{-8a}
Additional metabolic processes	TKT, IL1B, AR, CAT, GSS, NR3C1, SORD, NOX4, NOS1, ATF4, TNF, UGT1A1	54	3.37x10 ^{-12a}
Cell-to-cell signaling	IL1B, TF, AR, CREB1, IL6, GCK	6	7.59x10 ^{-3a}
Signal transduction	CCND1, FST, EGF, L1CAM, IL1B, AR, CAT, NR3C1, TNF, INSR, PTEN, PLCG1	29	8.84x10 ^{-3a}
Death	NR3C1, TNF, PTEN, SGK1, ADA, BCL2, HPRT1, IL6, SOD1, IL2, IL10, ALDH1A1	14	7.66x10 ^{-5a}
Additional biological processes	TKT, CCND1, FST, L1CAM, IL1B, TF, AR, GSS, NR3C1, NOX4, CSF3, NOS1	43	3.94x10 ^{-2a}

^aP<0.01.

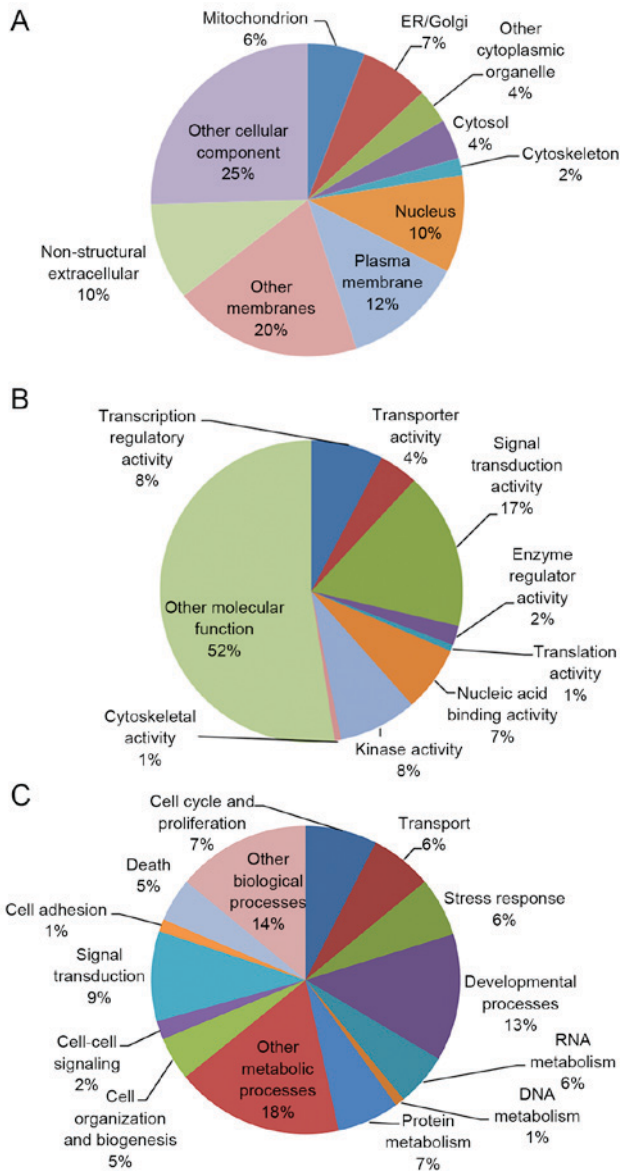


Figure 2. Gene ontology analysis of G6PD-associated genes. Each pie chart represents the (A) cellular components (B) molecular functions and (C) biological processes of G6PD-associated genes. G6PD, glucose-6-phosphate dehydrogenase; ER, endoplasmic reticulum.

GO analysis (Table II), and 98 G6PD-associated genes were identified and categorized according to cellular component, molecular function and biological process. The most highly represented categories of cellular components were ‘other cellular component’ (25%), ‘other membranes’ (20%) and the plasma membrane (12%; Fig. 2A). The most highly represented categories of molecular function were ‘other molecular function’ (52%), ‘signal transduction’ (17%) and ‘kinase activity’ (8%; Fig. 2B). The most highly represented categories of biological processes were ‘other metabolic processes’ (18%), ‘other biological processes’ (14%) and developmental processes (13%; Fig. 2C).

A gene network was constructed to identify a correlation between these 98 G6PD-associated genes, and the stability of gene regulatory networks (Fig. 3A). The most highly connected hub genes critically contribute to the stability of

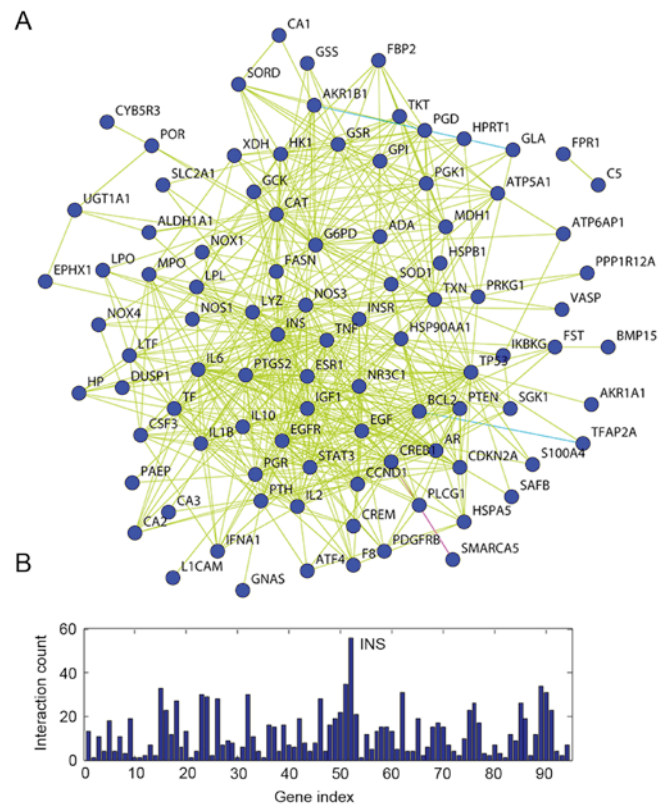


Figure 3. Network of G6PD-associated genes. (A) A schematic of the G6PD-associated gene network. The purple line indicates an experimentally confirmed connection, whereas the blue lines indicate a connection retrieved from the databases, and the yellow lines represent connections derived from literature mining. (B) Quantification of the connectivity of G6PD-associated genes. G6PD, glucose-6-phosphate dehydrogenase; INS, insulin.

the network. The most highly connected gene identified in the present study was *INS*, which encodes insulin that regulates glucose absorption (Fig. 3B).

The signaling pathways that correlated with each G6PD-associated gene were retrieved from the KEGG database, which implicated 47 pathways involved G6PD expression and activity. Out of these 7 were significantly associated with G6PD ($P < 0.01$; Table III). G6PD-associated genes were most significantly correlated with the cytokine-cytokine receptor interaction pathway. In addition, G6PD-associated genes were involved in regulating the mitogen-activated protein kinase (MAPK) signaling pathway, as well as the focal adhesion, Janus kinase (Jak)-signal transducer and activator of transcription (STAT), p53, long-term depression and apoptosis signaling pathways (Table III).

In addition to the notable PPP, G6PD-associated genes were observed to be involved in the regulation of fructose and mannose, glycerolipid, nitrogen, galactose, starch, sucrose, amino and nucleotide sugars, glutathione and drug metabolic pathways (3 or 4 counts for each metabolic pathway; $P<0.05$; Table IV). Furthermore, G6PD-associated genes were significantly correlated with a number of cancers, autoimmune diseases and oxidative stress-associated disorders (>3 counts for each disease; $P<0.05$; Table V). These results suggest that the aberrant regulation of G6PD signaling and/or metabolic

Table III. Signaling pathways significantly represented by glucose-6-phosphate dehydrogenase-associated genes.

Pathway ID	Pathway name	Genes	Count	Enrichment P-value
4060	Cytokine-cytokine receptor interaction	EGF, IL1B, CSF3, TNF, IFNA1, PDGFRB, EGFR, IL6, IL2, IL10	10	5.28x10 ^{-3a}
4010	MAPK signaling pathway	EGF, IL1B, ATF4, TP53, TNF, PDGFRB, EGFR, HSPB1, DUSP1, IKBKG	10	6.51x10 ^{-3a}
4510	Focal adhesion	CCND1, EGF, VASP, PTEN, PDGFRB, BCL2, EGFR, PPP1R12A, IGF1	9	2.75x10 ^{-3a}
4630	Jak-STAT signaling pathway	CCND1, CSF3, IFNA1, STAT3, IL6, IL2, IL10	7	8.11x10 ^{-3a}
4910	Insulin signaling pathway	INSR, HK1, INS, FBP2, GCK, FASN	6	1.67x10 ⁻²
4020	Calcium signaling pathway	NOS1, PLCG1, PDGFRB, EGFR, NOS3, GNAS	6	4.95x10 ⁻²
4115	p53 signaling pathway	CCND1, TP53, PTEN, IGF1, CDKN2A	5	3.30x10 ^{-3a}
4730	Long-term depression	NOS1, PRKG1, NOS3, IGF1, GNAS	5	3.98x10 ^{-3a}
4210	Apoptosis	IL1B, TP53, TNF, BCL2, IKBKG	5	9.85x10 ^{-3a}
4540	Gap junction	EGF, PDGFRB, EGFR, PRKG1, GNAS	5	1.13x10 ⁻³
4620	Toll-like receptor signaling pathway	IL1B, TNF, IFNA1, IL6, IKBKG	5	1.72x10 ⁻²
4660	T cell receptor signaling pathway	TNF, PLCG1, IL2, IL10, IKBKG	5	2.23x10 ⁻²
4920	Adipocytokine signaling pathway	TNF, STAT3, SLC2A1, IKBKG	4	1.75x10 ⁻²
4370	VEGF signaling pathway	PLCG1, HSPB1, PTGS2, NOS3	4	2.54x10 ⁻²
4640	Hematopoietic cell lineage	IL1B, CSF3, TNF, IL6	4	4.07x10 ⁻²
4612	Antigen processing and presentation	IFNA1, CREB1, HSPA5, HSP90AA1	4	4.22x10 ⁻²

^aP<0.01.

Table IV. Metabolic pathways significantly represented by glucose-6-phosphate dehydrogenase-associated genes.

Metabolic pathway	Genes	Count	Enrichment P-value
Pentose phosphate pathway	TKT, FBP2, GPI, PGD	4	5.42x10 ^{-4a}
Fructose and mannose metabolism	AKR1B1, SORD, HK1, FBP2	4	1.37x10 ^{-3a}
Glycerolipid metabolism	AKR1B1, GLA, LPL, AKR1A1	4	4.72x10 ^{-3a}
Starch and sucrose metabolism	UGT1A1, HK1, GCK, GPI	4	7.32x10 ^{-3a}
Nitrogen metabolism	CA1, CA2, CA3	3	5.22x10 ^{-3a}
Galactose metabolism	AKR1B1, HK1, GLA	3	5.87x10 ^{-3a}
Glutathione metabolism	GSS, GSR, PGD	3	3.83x10 ⁻²
Drug metabolism-other enzymes	UGT1A1, HPRT1, XDH	3	4.03x10 ⁻²
Amino sugar and nucleotide sugar metabolism	HK1, GCK, CYB5R3	3	2.60x10 ⁻²

^aP<0.01.

pathways may be associated with the pathogenesis of these diseases.

Analysis of miRNAs targeting G6PD. Four independent software programs were used to predict the miRNA-targets of G6PD. The searches yielded 33 miRNAs that were predicted by at least two software programs to target G6PD (Fig. 4). Out of these, three miRNAs, including miR-1207-5P, miR-1 and miR-125a-5p, were predicted to target G6PD by all four software programs, thus indicating that these miRNAs are most likely to be involved in the regulation of G6PD expression.

Discussion

A systematic analysis of G6PD-associated genes may provide useful information regarding the role of G6PD in healthy and pathological processes. In the present study, all genes reportedly associated with G6PD in published articles retrieved from the PubMed library were systematically analyzed. NLP and database searching strategies were employed to analyze the association between the identified genes and G6PD, as well as the signaling pathways that they may be involved in, and their potential connection to a number of clinical diseases.

Table V. Diseases significantly represented by glucose-6-phosphate dehydrogenase-associated genes.

Disease	Genes	Count	Enrichment P-value
Prostate cancer	CCND1, EGF, AR, ATF4, TP53, PTEN, PDGFRB, BCL2, EGFR, INS, CREB1, HSP90AA1, IGF1, IKBKG	14	2.81x10 ^{-11a}
Glioma	CCND1, EGF, TP53, PTEN, PLCG1, PDGFRB, EGFR, IGF1, CDKN2A	9	3.79x10 ^{-7a}
Melanoma	CCND1, EGF, TP53, PTEN, PDGFRB, EGFR, IGF1, CDKN2A	8	8.66x10 ^{-6a}
Small cell lung cancer	CCND1, NOS1, TP53, PTEN, BCL2, PTGS2, NOS3, IKBKG	8	3.61x10 ^{-5a}
Amyotrophic lateral sclerosis	CAT, NOS1, TP53, TNF, BCL2, SOD1, NOS3	7	1.46x10 ^{-5a}
Pancreatic cancer	CCND1, EGF, TP53, EGFR, STAT3, CDKN2A, IKBKG	7	8.63x10 ^{-5a}
Non-small cell lung cancer	CCND1, EGF, TP53, PLCG1, EGFR, CDKN2A	6	1.35x10 ^{-4a}
Alzheimer's disease	IL1B, NOS1, TNF, LPL, NOS3, ATP5A1	6	3.72x10 ⁻²
Bladder cancer	CCND1, EGF, TP53, EGFR, CDKN2A	5	3.65x10 ^{-4a}
Type II diabetes mellitus	TNF, INSR, HK1, INS, GCK	5	6.20x10 ^{-4a}
Endometrial cancer	CCND1, EGF, TP53, PTEN, EGFR	5	9.91x10 ^{-4a}
Colorectal cancer	CCND1, TP53, PDGFRB, BCL2, EGFR	5	8.13x10 ^{-3a}
Type I diabetes mellitus	IL1B, TNF, INS, IL2	4	3.69x10 ^{-3a}
Chronic myeloid leukemia	CCND1, TP53, CDKN2A, IKBKG	4	2.54x10 ⁻²
Graft-vs.-host disease	IL1B, TNF, IL6, IL2	4	3.10x10 ^{-3a}
Autoimmune thyroid disease	IFNA1, IL2, IL10	3	4.23x10 ⁻²

^aP<0.01.

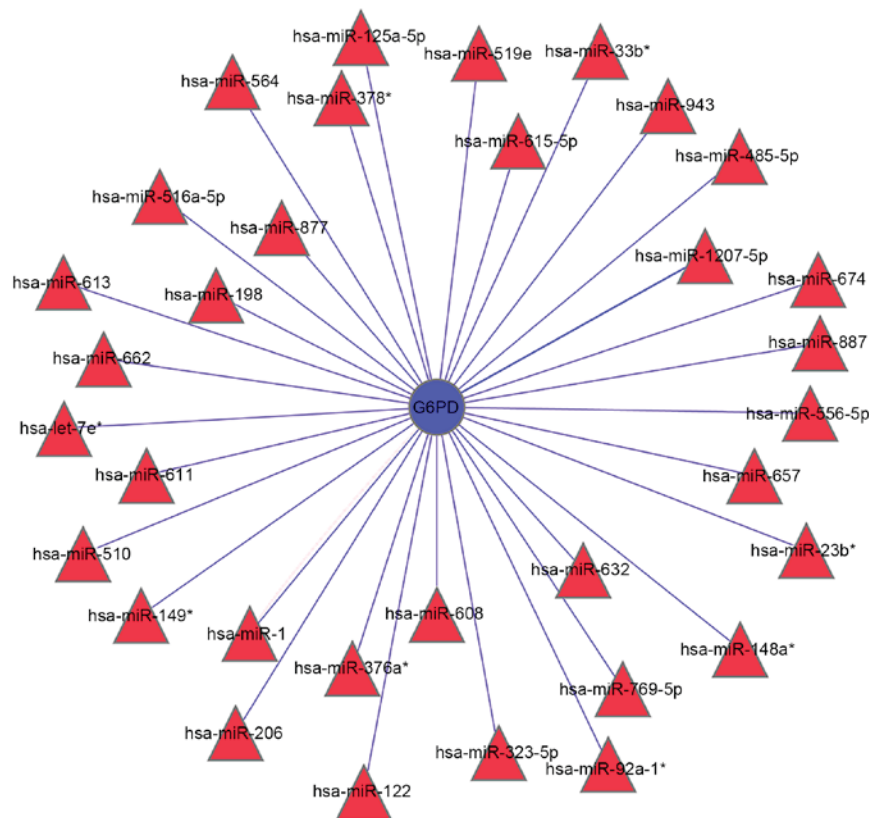


Figure 4. Integrative analysis of miRNAs predicted to target G6PD. A total of 33 miRNAs were identified to potentially modulate the expression and/or activity of G6PD using four miRNA target prediction software programs. miRNA, microRNA; G6PD, glucose-6-phosphate dehydrogenase. ^aThe lower expression quantity product by the same precursor of miRNA.

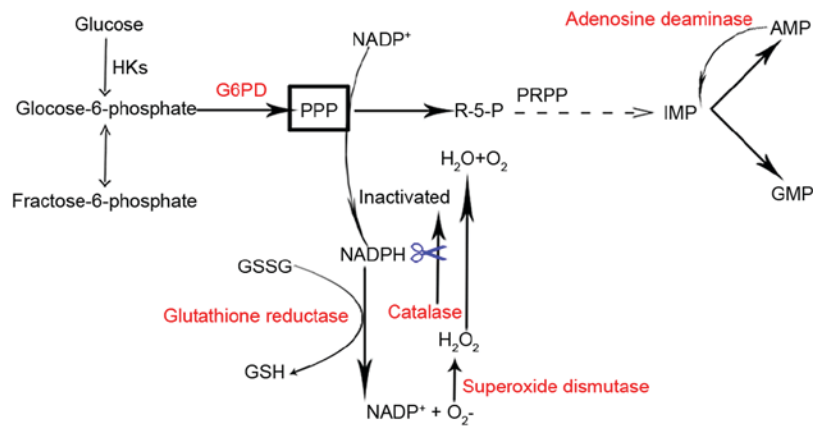


Figure 5. A schematic indicating the enzymatic reactions that involve the most cited G6PD-associated genes. As the rate-limiting enzyme in the pentose phosphate pathway, G6PD catalyzes the production of R-5-P and NADPH. R-5-P is converted to IMP, which leads to the production of AMP. This product is converted to IMP when adenosine deaminase is present. NADPH serves crucial roles in the reduction of GSSG to GSH by glutathione reductase, and protects catalase from inactivation by H_2O_2 . HKs, hexokinases; G6PD, glucose-6-phosphate dehydrogenase; R-5-P, ribose-5-phosphate; PPP, pentose phosphate pathway; $NADP^+$, oxidized nicotinamide adenine-dinucleotide phosphate; NADPH, reduced nicotinamide adenine-dinucleotide phosphate; PRPP, phosphoribosyl pyrophosphate; IMP, inosine monophosphate; AMP, adenosine monophosphate; GMP, guanosine monophosphate; GSSG, glutathione disulfide; GSH, reduced glutathione.

A total of 98 G6PD-associated genes were identified, and out of the 47 implicated signaling pathways, seven were highly likely to be regulated by G6PD-associated genes. These results highlight the mechanisms by which G6PD dysregulation may contribute to cancer or autoimmune diseases. In addition, four separate databases were used to identify 33 miRNAs that were predicted to regulate G6PD. To the best of the author's knowledge, this is the first study to perform systematic analysis of miRNAs that may target G6PD.

Out of the 98 G6PD-associated genes that were identified, the five most frequently cited genes were GSR, INS, CAT, SOD1 and ADA. The products encoded by these genes are involved in maintaining homeostasis of NADPH. GSR utilizes NADPH, derived from the PPP (of which G6PD catalyzes the first committed step), for recycling oxidized glutathione disulfide to reduced glutathione (Fig. 5) (53,54). In addition, NADPH is required and consumed during fatty acid synthesis. Reduced consumption of NADPH has been observed when fatty acid synthesis is inhibited by AMP-activated protein kinase (AMPK) via inhibition of acetyl-coenzyme A carboxylase (55). The INS gene encodes insulin, which increases cell permeability to fatty acids and accelerates the pentose phosphate cycle for NADPH production in the liver (56,57). The CAT gene encodes catalase, a crucial scavenger of hydrogen peroxide in human erythrocytes, which is dependent on the availability of PPP-derived NADPH (58). NADPH is known to be tightly bound to mammalian catalase, and prevents inactivation of catalase by H_2O_2 (Fig. 5) (59). Significantly lower SOD and G6PD activities have been reported in cystic echinococcosis of the liver and Parkinson's disease (57,60). Reduced SOD activity in erythrocytes, potentially as a result of inactivation of SOD by diminished reductive equivalents, has been detected in 26 carriers of G6PD defects (Fig. 5) (61).

Signaling pathway analysis identified 47 pathways, of which the cytokine-cytokine receptor interaction was most significantly associated with G6PD-related genes. In addition, G6PD-associated genes were demonstrated to be potentially involved in regulating focal adhesion, the MAPK, Jak-STAT,

p53, long-term depression and apoptosis signaling pathways. Notably, G6PD itself may be involved in regulating the MAPK and p53 signaling pathways. By increasing the availability of glucose for glycolysis, AMPK functions as a master sensor of cellular energy balance. AMPK is activated when cells undergo energy-depleting stresses (62-65). Due to insufficient NADPH, G6PD-deficiency leads to a switch of the biosynthetic pathway for GSH, thus limiting AMPK activation (62). p38 MAPK has been implicated in a number of cellular processes, including the cell cycle, apoptosis, differentiation, neoplasm metastasis, angiogenesis and vasculogenesis (63). Transforming growth factor- β -activated protein kinase 1-binding protein 1 (TAB1) is a scaffold protein responsible for the autophosphorylation of MAPK (64). Activation of AMPK has been demonstrated to recruit p38 MAPK to the TAB1/AMPK-containing macromolecular complex, thus facilitating p38 MAPK activation in the ischemic heart (64).

It was previously reported that p53 might prevent the inactive G6PD monomer from forming active dimers (65). In addition, p53 may be regulated by G6PD. Following phosphorylation by ataxia telangiectasia mutated (ATM), p53 regulates lipid metabolism through promoting transcription of Lpin1, which encodes a protein that regulates lipid metabolism (66,67). Notably, Lpin1 induction is dependent on elevated ROS and ATM levels, and this process is inhibited by the N-acetyl cysteine antioxidant (66). ATM is activated directly by oxidative stress (67). Considering that the ROS-ATM-p53 signaling pathway contributes to the induction of Lpin1 and G6PD greatly influences ROS levels, it is reasonable to hypothesize that G6PD may regulate p53 function. Whether G6PD regulates MAPK and p53 signaling pathways remains to be addressed in future studies.

Alteration of G6PD-regulated pathways may lead to certain diseases. Dysregulation of G6PD has been reported to be associated with neoplasms (68-70). For example, increased G6PD expression levels correlated with grading, metastasis and poor prognosis in human hepatocellular carcinoma patients (68). Alterations in G6PD levels may promote tumor cell proliferation

or apoptosis via the STAT3/5 pathway in a human melanoma xenograft model, and G6PD regulated STAT3 activity via the NADPH oxidase 4-NADPH-ROS-*proto-oncogene* tyrosine protein kinase/protein-tyrosine phosphatase 1D signaling pathway in A375 melanoma cells (69,70). In the present study that G6PD-associated genes were highly correlated with various cancers, including prostate cancer, glioma, small cell lung cancer, non-small cell lung cancer, chronic myeloid leukemia, pancreatic cancer and bladder cancer. In addition, G6PD-associated genes were highly correlated with autoimmune diseases, including amyotrophic lateral sclerosis, graft-versus-host diseases, and autoimmune thyroid disease. One of the G6PD-associated genes, interleukin 6 (IL6), has been reported to be involved in autoimmune diseases (71). Activation of the IL6 inflammatory loop may mediate trastuzumab resistance in human epidermal growth factor receptor 2-positive breast cancer by promoting growth of the cancer stem cell population (72).

Accumulating evidence suggests that miRNAs are involved in various biological processes and human diseases, and they are versatile regulators of gene expression in higher eukaryotes and additional organisms. Aberrant expression of G6PD leads to the development of a number of different diseases. Therefore, miRNAs capable of targeting G6PD may be of therapeutic interest. Previous studies have reported the potential for particular miRNAs to influence G6PD expression (73,74). Therefore, the aim of the present study was to identify all miRNAs capable of binding G6PD. A total of 33 miRNAs were predicted to target G6PD in at least two of four independent programs employed in the current study. miR-1207-5P, miR-1 and miR-125a-5p were the only three miRNAs predicted by all four software programs to potentially target G6PD, indicating that these three miRNAs may be potentially important regulators of G6PD. Notably, Alvarez (75) validated G6PD as a direct target of miR-1207-5p using a dual Firefly-*Renilla* luciferase reporter assay.

In conclusion, the present study systematically evaluated G6PD-associated genes and miRNAs that potentially target G6PD. The results of the analysis identified signaling and metabolic pathways regulated by G6PD-associated genes, as well as miRNAs that are predicted to target G6PD. This may provide a greater understanding of the role of G6PD in different pathological processes, and contribute to the development of rational therapeutic approaches for G6PD-associated diseases. However, further experiments will be required to confirm the results. By integrating genomics, transcriptomics, and proteomics analyses of G6PD-associated disorders, a novel area of G6PD research may be explored.

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