MOLECULAR BASIS OF G6PD DEFICIENCY: CURRENT STATUS AND ITS PERSPECTIVE

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Abstract- Glucose-6-phosphate dehydrogenase is an essential enzyme to cell growth. Its deficiency of enzyme plays an important role in senescence and death signaling. Also, it is actually the most common clinically important enzyme defect, not only in hematology, but also among all human known diseases. Clinical consequences of enzyme deficiency are: neonatal hyperbilirubinemia, acute hemolytic anemia, and chronic hemolytic anemia. The enzyme gene spans 18 kb on the X chromosome (xq28) and contains 13 exons. Its promoter is embedded in a CpG island that is conserved from mice to humans. The development of a number of PCR-based methods for the detection of known mutations in Glucose-6-phosphate dehydrogenase has made it possible to detect enzyme deficiency and identify the specific mutation responsible with relative ease. We will discuss the mentioned clinical manifestations of glucose-6-phosphate dehydrogenase deficiency, Genetics, biochemistry and pathophysiology of the enzyme in details using newer published data and present most of the studies in Iranian population.

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INTRODUCTION

Glucose 6 phosphate dehydrogenase (G6PD) deficiency was discovered in 1950s, and shown to be the cause of hemolytic effect of primaquine (1). It soon became apparent that G6PD deficiency was a widespread genetic defect and hereditary deficiency of G6PD turned out to be among the most common genetic disorders, affecting more than 400 million people worldwide. G6PD deficiency is actually the most common clinically important enzyme defect, not only in hematology, but also among all human diseases known (2). The Glucose 6 phosphate dehydrogenase (G6PD) deficiency and the enzyme itself are studied in detail and epidemiologic studies are available for many parts of the world.

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In this article we will discuss the disease and the enzyme in detail using newer published data and present most of the studies in Iranian population.

EPIDEMIOLOGY

The genetic abnormality is distributed world wide. Areas of high prevalence are Africa, Southern Europe, the Middle East, South-East Asia and Oceania. In the Americans and in parts of Northern Europe G6PD deficiency is also quite prevalent as a result of migrations in relatively recent historical times. Although accurate quantitative data are lacking, fava beans are probably still today the most common trigger of hemolysis in G6PD-deficient subjects: therefore the incidence of this clinical manifestation can be identified with the epidemiology of favism. Fava beans are grown world-wide; they are a significant component of the diet particularly in the Middle East, in Iran and in Southern Europe (3).

About 7.5% of the world population are carriers of G6PD deficiency ranging from 35% in Africa to 0.1% in Japan and some European areas (4). Although the literature on the epidemiology of G6PD deficiency in the Middle East is quite extensive, there are some papers in this regard in Iranian populations. In the past 8 years Dr. M.R Noori Daloii and his colleagues have conducted extensive studies on the molecular genetics of G6PD in Iranian populations which indicate 9.8 percent G6PD deficiency among different provinces and tribes (4-11). Molecular analysis of G6PD deficiency in Mazandaran province revealed three major polymorphic variants: G6PD Mediterranean (C563T, Ser 188 Phe) 66.2%, G6PD Chatham (G1003A, Ala 335 Thr) 27%, and G6PD Cosenza (G1367C, Arg 459 Pro) 6.75%. These findings indicate a higher prevalence of G6PD Chatham in this population than anywhere else in the world (5, 6). Results of a study performed in Gilan province was: G6PD Mediterranean 86.4%, G6PD Chatham 9.71%, but none of samples were found to have Cosenza mutation. It is interesting that the Chatham mutation does not have high frequency when compared with Mazandaran (7). A study in Golestan province showed that the Mediterranean mutation was predominant in this province, as in the case of Mazandaran and Gilan, (69%), 26.7% of patients had Chatham mutation, but non of them had Cosenza mutation (8). When researchers pooled data of these three provinces (Mazandaran, Gilan, and Golestan) resulting allele frequencies for Mediterranean, Chatham, and Cosenza became 70.78%, 18.35%, and 1.78% respectively (4). In Khorasan province 66% of patients had Mediterranean mutation, 12% had Chatham mutation, but Cosenza mutation was not detected (9, 10). Results of Sistan and Balochestan province are: G6PD Mediterranean 80.42%, G6PD Chatham 2.17%, and G6PD Cosenza was not reported (11). Hormozgan province had 79.45% G6PD Mediterranean, 8.21% G6PD Chatham, and 12.33% G6PD Cosenza (10). Allele frequencies in Fars province were 84.62% Mediterranean mutation, 12.82% Chatham mutation, 1.28% G6PD A^-, 1.28% G6PD Canton. In this study G6PD Cosenza was not found (12). In Kermanshah province the most common allele was found to be the G6PD Mediterranean (91.2%), followed by the G6PD Chatham (7.3%), and the G6PD Cosenza (1.5%). These findings indicate that the allele frequency of G6PD Mediterranean mutation in Kermanshah is higher than those from two Fars ethnic groups living in Northern and Southern Iran. (13). All of these studies show that G6PD Mediterranean is the most prevalent allele in Iranian populations, which is the same in other countries like Turkey, Italy, Pakistan, India, Bahrain, Kuwait, Oman, Saudi Arabia, Iraq, and Greece (8).

CLINICAL MANIFESTATIONS

Neonatal hyperbilirubinemia

Neonatal hyperbilirubinemia (neonatal jaundice) is one of the most health-threatening consequences of G6PD deficiency (14, 15). The prevalence of the disease is twice that of the general population (16) in males who carry the defective gene and in homozygous females. It rarely occurs in heterozygous females (17, 18). The mechanism by which G6PD deficiency causes neonatal hyperbilirubinemia is not fully understood. Although hemolysis may be observed in neonates who have G6PD deficiency and are jaundiced (19), other mechanisms appear to play a more important role in the development of hyperbilirubinemia (20-22). Hyperbilirubinemia is likely secondary to impairment of bilirubin conjugation and clearance by the liver leading to indirect hyperbilirubinemia (20, 21). Infants with G6PD deficiency and a mutation of uridine diphosphoglucuronate glucuronosyltransferase 1 gene promoter (UDPGT-1; which is the enzyme affected in Gilbert disease) are particularly susceptible to hyperbilirubinemia secondary to decreased liver clearance of bilirubin (24).

Acute hemolytic anemia (AHA)

G6PD-deficient subjects are at risk of developing AHA in response to three types of triggers: (i) fava beans, (ii) infections, and (iii) drugs. Typically, a hemolytic attack starts with malaise, weakness, and abdominal or lumbar pain. After an interval of several hours to 2-3 days the patient develops jaundice and dark urine, due to hemoglobinuria. The

onset can be extremely abrupt, especially with favism in children. The anemia is from moderate to extremely severe, it is usually normocytic and normochromic and it is due largely to intravascular hemolysis: hence it is associated with hemoglobinemia, hemoglobinuria and low or absent plasma haptoglobin. A classical test, now rarely carried out, is supravital staining with methyl violet which, if done promptly, reveals the presence of Heinz bodies, consisting of precipitates of denatured hemoglobin, and regarded as a signature of oxidative damage to red cells (except for the rare occurrence of an unstable hemoglobin). The most serious threat from AHA in adults is the development of acute renal failure (this is exceedingly rare in children). Once the threat of acute anemia has passed, and in the absence of co-morbidity, full recovery form AHA associated with G6PD deficiency is the rule (3). Hemolysis occurs after exposure to the stressor but does not continue despite continued infection or ingestion. This is thought to be a result of older erythrocytes having the greatest enzyme deficiency and undergoing hemolysis first. Younger erythrocytes and reticulocytes that typically have higher levels of enzyme activity are able to sustain the oxidative damage without hemolysis (25).

Favism

The fact that exposure to fava beans (Vicia fava, broad bean) is toxic and potentially fatal for some individuals has been known since the time of the old Greeks. Favism is the acute hemolysis that follows the ingestion of fava beans. The syndrome seems to be restricted to those having the Mediterranean variant with more frequency among males than females. There is an increased incidence between the ages of 2 and 6 years. Favism can even occur in the breast-fed infant whose mother had ingested fava beans. The haemolytic crisis of favism is in all respects similar to that triggered by drugs (26). Patients with favism are always G6PD deficient, but not all G6PD-deficient individuals develop hemolysis when they ingest fava beans. Thus, G6PD deficiency is a necessary but not sufficient cause of favism. Favism is the most common in persons with G6PD class II variants, but rarely can it occur in patients with the G6PD A– variant (27). Fava beans are presumed to cause oxidative damage by an unknown component, possibly vicine, convicine, or isouramil (25, 28). As fava beans are a common food, especially in northern Iran, favism is also very common in this area (5), actually from a clinical point of view, favism is the most common manifestation of G6PD deficiency in Iran (4).

Drug-induced hemolysis

G6PD deficiency was discovered as a result of a series of investigations performed to understand why some persons were uniquely sensitive to the development of hemolytic anemia when they ingested antimalarial drug primaquine (29). Thus, the first and best-known morbid effect of G6PD deficiency was drug-induced hemolysis. Primaquine is one of many drugs that shortens RBC life span in G6PD-deficient persons (30). Drugs that cause hemolysis in G6PDdeficient persons inflict oxidative damage to erythrocytes leading to erythrocyte destruction. Hemolysis typically occurs 24 to 72 hours after ingestion, with resolution within four to seven days (31).

Oxidative drugs ingested by a woman who is breastfeeding may be transmitted in breast milk and can cause acute hemolysis in a G6PD deficient child (17,32).

Infection-induced hemolysis

Although, for historical reasons, drug-induced hemolysis has attracted the most attention, it is likely that hemolysis induced by infection may be a more common cause of clinically significant hemolysis (33-35).

Although the exact mechanism by which this occurs is unknown.

Leukocytes may release oxidants during phagocytosis that cause oxidative stress to the erythrocytes; however, this explanation alone would not account for the variety of infections associated with hemolysis in G6PD-deficient persons. The most common infectious agents causing hemolysis include Salmonella, Escherichia coli, beta-hemolytic streptococci, rickettsial infections, viral hepatitis, and influenza A (26).

Chronic hemolytic anemia

In rare cases, G6PD deficiency results in (usually mild) chronic haemolysis and anaemia (hereditary non-spherocytic haemolytic anaemia). The patient is always a male, almost invariably develops neonatal jaundice, and in general he is investigated because of that or because of unexplained jaundice or because of gallstones later in life. Usually the spleen is moderately enlarged in small children, and subsequently it may increase in size sufficiently to cause mechanical discomfort, or hyper splenism, or both. The severity of anemia ranges in different patients from borderline to transfusion dependent. The anemia is usually normochromic but somewhat macrocytic, largely on account of reticulocytosis (up to 20 percent or more). The red-cell morphology is not characteristic (hence the designation non-spherocytic). Bilirubin and LDH are increased. The bone marrow is normoblastic, unless there is superimposed folate deficiency. The variant that causes chronic haemolysis is uncommon because it is related to sporadic gene mutation (class 1 or class 2) rather than the more common inherited gene mutation (36). Presumably class 1 or 2 variants produce chronic hemolysis because the functional severity of the defect is so great that the erythrocyte cannot even withstand the normal stresses that it encounters in the circulation. The functional severity in these patients is not usually reflected by the level of the enzyme as it is measured in the laboratory. The most consistent common feature of these variants is the location of the mutation. In the great majority of cases, it is in the region of the putative NADP-binding or glucose-6-phosphate binding site of the molecule. So possibly the disease is caused by susceptibility to inhibition by NADPH or in vivo liability (37, 38).

PATHOPHYSIOLOGY

The enzymatically active form of G6PD (D-glucose-6-phosphate: NADP 1-Oxidoreductase), is either a dimer or a tetramer of a single protein subunit of 514 amino acids with a molecular mass of 59096 Da and contains tightly bound NADP (39, 40). Aggregation of the inactive monomers into catalytically active dimers and higher forms requires the presence of NADP (41). Thus, NADP appears to be bound to the enzyme both as a structural component and as one of the substrates of the reaction (39, 42 and 43). Some regions of the molecule critical for its functions have been identified because they are highly conserved in evolution. The G6P-binding site and the active center of the enzyme are located near lysine 205. In the dimer structure the two subunits are symmetrically located across a complex interface of B-sheets. The NADP binding site is near the N-terminus, and bound NADP is important for the stability of G6PD (3).

G6PD catalyzes the first step in the hexose monophosphate pathway (HMP). It oxidizes glucose-6-phosphate to 6-phosphogluconolactone, reducing NADP to NADPH. The HMP is the only source of NADPH in the erythrocytes and it also serves to produce the ribose needed for synthesis of nucleotides in the salvage pathways. The main function of the pathway seems to be to protect the RBC against oxidative damage (44). NADPH provided in this way is required for the reduction of oxidized glutathione to the reduced state of glutathione (GSH), as well as for the reduction of mixed disulphides of glutathione and cellular proteins (23). GSH, in turn, is necessary for the removal of red cell oxidants, such as superoxide anion (O^{2-}) and hydrogen peroxide (H_2O_2). This is important, since the accumulation of oxidants may reduce the life span of the erythrocyte by accelerating the rate of oxidation of erythrocyte proteins including haemoglobin, plasma membrane protein(s) and enzymes. Studies using G6PD knock out mice, demonstrating an increased sensitivity towards H_2O_2 and towards sulphhydryl group oxidizing agents, emphasizing the important role of G6PD in the defense against oxidative stress (45). Because erythrocytes do not generate NADPH in any other way, they are more susceptible than other cells to destruction from oxidative stress (46). The level of G6PD activity in affected erythrocytes generally is lower than in other cells (47). Normal red blood cells that are not under oxidative stress generally exhibit G6PD activity at approximately 2 percent of total capacity (48). Even with enzyme activity that is substantially reduced, there may be few or no clinical symptoms. A total deficiency of

G6PD is incompatible with life (27) because Glucose 6-phosphate dehydrogenase (G6PD) is a housekeeping enzyme critical in the redox metabolism of all aerobic cells.

GENETICS

G6PD was cloned and sequenced by Persico *et a1.* (47-49) and then independently by Takizawa and Yoshida (50). The G6PD gene spans 18 kb on the X chromosome (xq28) and contains 13 exons. The G6PD promoter is embedded in a CpG island that is conserved from mice to humans (51, 52). The promoter of the G6PD gene contains a TATA-like sequence, TTAAAT, and numerous stimulatory protein 1 (Sp1) elements, but no CAAT element (53-56). S1 nuclease and primer extension analyses of mouse G6PD mRNA indicate that the transcriptional start site used in liver and adipose tissue, in which lipogenesis is regulated, is the same as in kidney, in which G6PD is expressed constitutively (57).These results indicate that the 5´-end of the mRNA is the same in all cell types. Three DNase I hypersensitive sites have been localized in the 5´-end of the G6PD gene. Hss-1 (-1000 bp) and Hss-2 (-400 bp) are present in all tissues, but Hss-3, which is located in intron 2 (+1500 bp), is liver specific (57).The translation start site is located in exon 2 and has been mapped in rats, mice, and humans (58-60). The number of exons and introns and the size and sequence of the exons are conserved in higher eukaryotes. The sequence similarity between the human G6PD cDNA and that of mice or rats is 87%. The similarity between the mouse and rat cDNA sequences is even greater (93% identity). Most of the dissimilarity is in the 3´-untranslated region (3´-UTR). Exon 13 is at least 800 nucleotides long and contains the translation stop codon. The 3´-UTR is approximately 600 nucleotides long and contains a single polyA site. The structure of the gene is unusual in that the second intron is 11 kb and accounts for almost half of the gene; the large size of this intron is also conserved between humans, rats, and mice. The amount of G6PD activity and mRNA abundance differs between tissues (61, 62) and most likely reflects tissue-specific differences in growth rate, reductive biosynthetic reactions, and oxidative stress. Moreover, G6PD expression is regulated by hormonal and nutritional factors in only a few tissues. G6PD expression is regulated in liver and adipose tissue, and its activity correlates with the rate of fatty acid biosynthesis. It is also induced in lactating mammary glands by dietary carbohydrate (62).

The X-linkage of the G6PD gene has important implications. First, as males have only one G6PD gene (i.e. they are hemizygous for this gene), they must be either normal or G6PD-deficient. By contrast, females, who have two G6PD genes, can be either normal or deficient (homozygous), or intermediate (heterozygous). Moreover, as a result of the phenomenon of X-chromosome inactivation, heterozygous females are genetic mosaics, and this in turn has clinical implications. Indeed, in most other (autosomal) enzyme deficiencies, heterozygotes are asymptomatic because cells with an enzyme level close to 50 percent of normal are biochemically normal. However, in the case of G6PD, as a result of X-inactivation, the abnormal cells of a woman heterozygous for G6PD efficiency are just as deficient as those of a hemizygous deficient man, and therefore just as susceptible to pathology (3).

Nature of mutations

G6PD-deficient subjects have invariably been found to have mutations in the coding region of the G6PD gene. The current database of some 140 mutants consists, with few exceptions, of single missense point mutations, entailing single amino acid replacements in the G6PD protein. The exceptions are small deletions (of one to eight amino acids), and a few instances in which two point mutations rather than one are present (for instance, in G6PD A-b the variant most commonly encountered in Africa). All these three types of mutations are found in multiples of three nucleotides so that a frameshift does not occur. Only one splicing mutation has been found and no promoter mutations have been identified. In most cases these mutations cause G6PD deficiency by decreasing the in vivo stability of the protein: thus, the physiological decrease in G6PD activity that takes place with red cell aging is greatly

accelerated. In some cases an amino acid replacement can also affect the catalytic function of the enzyme (63).

Balanced polymorphism and malaria selection

When a gene that has some potential for decreasing fitness achieves a high frequency in some populations, it is necessary to assume that in those populations it also confers a survival advantage. Thus, a balance has been achieved between the advantage and the disadvantage conferred by a gene, and this is designated a balanced polymorphism. One of the most studied of such polymorphisms is the mutation for sickle Hb, and evidence from a variety of sources has led to the conclusion that the advantage conferred by this gene is resistance to falciparum malaria.

The mortality caused by malaria in some parts of the world is so high that a large number of genetic traits that defend against this infection have evolved in mankind, and many polymorphisms affecting the RBC seem to have reached high frequencies for this reason (64).

The geographic distribution of G6PD deficiency (see figure) led researchers to suggest that G6PD deficiency is also one of the polymorphisms that confers resistance to heterozygotes, and perhaps to hemizygotes as well, against infection with falciparum malaria. The evidence for this, reviewed in detail by Greene (65).

World distribution of polymorphic G6PD-deficient mutants. The different shadings indicate the frequency of the G6PD deficienct phenotype in the respective population. Modified from Vulliamy T, Luzzatto L. (2003).

DIAGNOSIS

Screening for G6PD deficiency

In hemizygous males who are not undergoing hemolysis, as will be found in population surveys, semi-quantitative or nonquantitative screening methods are entirely adequate. Dye reduction tests, first introduced by Motulsky and Campbell-Kraut as the brilliant cresyl blue decolorization test, have been widely used (66). Although this test is still sometimes used, particularly in population surveys, it has largely been replaced by the fluorescent spot test, in which the generation of NADPH is detected directly visually under ultraviolet light (67-69) and nowadays fluorescent spot test is the most popular test used in this purpose (25).

Detection of G6PD deficiency in patients undergoing hemolysis

In patients with acute hemolysis, testing for G6PD deficiency may be falsely negative because older erythrocytes with a higher enzyme deficiency have been hemolyzed. Young erythrocytes and reticulocytes have normal or near-normal enzyme activity (70, 71). Several different approaches may be used to diagnose patients who have just undergone hemolysis. The simplest is merely to wait for a week or two or to perform family studies. Alternatively, one may deplete the sample being studied of reticulocytes by centrifugation (72). Another approach is to compare the activity of G6PD with that of another age-dependent RBC enzyme such as hexokinase or glutamic oxaloacetic transaminase. The most powerful approach for establishing the diagnosis in the context of hemolysis is analysis of genomic DNA obtained from circulating leukocytes. Neither the presence of young erythrocytes nor, for that matter, of transfused cells confounds the results obtained from such an analysis (72).

Heterozygote detection

Female heterozygotes may be hard to diagnose of X-chromosome inactivation (73-75). Heterozygotes have two RBC populations one of these populations consists of normal RBCs and the other of RBCs that are as deficient as those of a hemizygous male with

the same deficient variant. On the average, half of the cells are normal and half are deficient. However, in some heterozygous women most of the cells are deficient; in others most are normal. The result of assaying the activity of enzyme per gram Hb reflects the proportion of normal and abnormal cells in the individual being studied, and some heterozygous women will have normal RBC enzyme activity whereas others will be grossly deficient in enzyme activity. Thus, the usual RBC enzyme activity measurements cannot be relied upon for the detection of heterozygotes. A more acceptable approach is to use techniques in which each RBC acts as an independent metabolic unit. Methemoglobin reduction, nile blue sulfate (76, 77), and reduction of a tetrazolium dye (78, 79) are newer techniques to solve the problem. Although such methods may be able to identify heterozygotes with as few as 5% to 10% normal or abnormal cells, some heterozygotes will escape detection because virtually no normal or no abnormal cells are present in the circulation. The most accurate method for heterozygote detection is to detect the mutation in genomic DNA. Although X-inactivation may alter the methylation pattern on the inactive X-chromsom (80, 81) and prevent transcription of the inactive gene (82), it does not prevent the detection of the difference in the nucleotide sequence of the gene. Thus, heterozygote detection by DNA analysis is entirely reliable, provided that the mutation to be detected is known.

Identification of G6PD variants

It became apparent early in the study of G6PD deficiency that there were differences in the characteristics of the residual enzyme in different deficient individuals. Fortunately, a WHO expert committee standardized the methods for the purification and characterization of G6PD variants in 1967 (83) and most investigators subsequently used the same techniques for the examination of different variants. The technology was based on purifying the enzyme followed by ammonium sulfate fractionation. The enzyme is then examined kinetically, electrophoretically, and by measuring its thermal stability. Limitations of the techniques is the fact that the volumes of blood required were large, and it was often difficult to be certain whether relatively minor differences in properties were caused by the existence of new variants or whether the observed variation was methodologic, *e.g*. G6PD Cornel1 and Chicago, were shown to be from members of the same extended family (83).

The development of a number of PCR-based methods for the detection of known mutations in G6PD has made it possible to detect G6PD deficiency and to identify the specific mutation responsible with relative ease. The advantage of the use of this type of technology is that DNA samples are much more stable than the enzyme in blood samples, and that very small volumes suffice for diagnosis. Methods of detection include the use of restriction endonucleases to cleave naturally occurring restriction sites (84) or restriction sites produced by making mismatched oligonucleotides (85, 86) and allele-specific oligonucleotide hybridization. These methods are sufficiently facile for population screening and require so small a sample that they can be used for prenatal diagnosis (86).

Who should be tested?

G6PD deficiency is one of a group of congenital hemolytic anemias, and its diagnosis should be considered in children with a family history of jaundice, anemia, plenomegaly, or cholelithiasis, especially in those of Mediterranean or African ancestry (87). Testing should be considered in children and adults (especially males of African, Mediterranean, or Asian descent) with an acute hemolytic reaction caused by infection, exposure to a known oxidative drug, or ingestion of fava beans. Although rare, G6PD deficiency should be considered as a cause of any chronic nonspherocytic hemolytic anemia across all population groups. The World Health Organization recommends screening all newborns in populations with a prevalence of 3 to 5 percent or more in males (44).

TREATMENT

The main treatment for G6PD deficiency is avoidance of oxidative stressors. Folic acid and iron potentially are useful in hemolysis, although G6PD

deficiency usually is asymptomatic and the associated hemolysis usually is short-lived. (22, 88) Research is being done to identify medications that may inhibit oxidative induced hemolysis of G6PD-deficient red blood cells (89).

NEW ASPECTS

G6PD, aging and longevity

NADPH produced by G6PD is a critical modulator of the cellular redox state and serves as a reductant for several enzymes which generate oxygen-free radicals, including the leukocyte NADPH oxidase (91) and its more widely distributed homolog Nox1 (92), nitric oxide synthase, the cytochrome P450 monooxygenases (92), and the Fenton reaction of iron-mediated catalysis of hydroxyl radical formation from H_2O_2 (93). Thus, a reduction in the supply of NADPH (which happens in G6PD deficiency) could have a profound effect on oxygen-free radical production. Oxidative stress and associated inflammatory processes are believed to play an important role in the pathogenesis of major age-related diseases, such as cancer (94), atherosclerosis (95), and Alzheimer's disease (96, 97), as well as the basic aging process. Studies in Sardinian males bearing the Mediterranean variant of G6PDH deficiency, both with cells *in vitro* and in epidemiologic observations, support the hypothesis that reduced G6PDH activity has a beneficial effect on age-related disease development and longevity. Recent epidemiological studies have found that G6PD deficient Sardinian males have a reduced mortality from cerebrovascular and cardiovascular disease and are more likely to achieve centenarian status than their normal counterparts (97, 98).

G6PD and cell growth

A number of studies suggest that G6PD is essential to cell growth. Using a putative inhibitor of G6PD dihydroepiandrosterone (DHEA), Tian *et al*. showed that suppression of G6PD activity led to diminished proliferation of several cell lines (99). It is controversial if DHEA inhibits G6PD activity in cultured cells. Previous studies had demonstrated that DHEA could inhibit the activity of a purified G6PD preparation (100). However, it appears that DHEA and analogs do not exert a long-term inhibitory effect on G6PD activity in cultured cells: G6PD activity dropped transiently after DHEA treatment and returned to normal several hours later. Similar kinetics of G6PD inhibition was observed in erythrocytes.

G6PD and cell senescence

As mentioned before, the involvement of reactive oxygen species in cellular senescence is not unprecedented. Reactive oxygen species (ROS) produced during metabolism cause cumulative damage, resulting in senescence. Owing to the imperfect nature of respiration, roughly 1-2% of electron flow contributes to chemical reduction of O^2 to $\mathbf{O^{2\bullet-}}$, which is sequentially converted to other ROS, such as hydrogen peroxide and hydroxyl radical. These ROS are known to damage proteins, lipids, mitochondrial DNA and genomic DNA in a relatively indiscriminate manner. As this damage accumulates, the ability of cells to grow is ultimately impaired, provoking senescence. This view is supported by studies with *Drosophila* strains overexpressing Cu/Zn superoxide dismutase and/or catalase, where significantly extended mean and maximum lifespans were observed (101), and studies in which exogenous SOD1 expression in the **motor neurons** of *Drosophila* increased the organism's normal lifespan by up to 40% (102).

As G6PD is indispensable to maintenance of the redox balance and detoxification of ROS, it is likely that G6PD deficiency cripples the antioxidant defense, resulting in the buildup of oxidative damage and thus cellular senescence. Consistent with this notion G6PD-deficient cells had lower intracellular G6PD activity and NADPH/NADP+ ratio but higher level of 8-hydroxydeoxyguanosine (8-OHdG) compared with normal counterparts (103).

The redox status is increasingly tilted towards the oxidizing end during their serial passage. This correlates well with their tendency to undergo senescence. Moreover, G6PD-deficient cells display increased propensity for $\mathbf{H_2O_2}$-induced senescence. These findings suggest the involvement of ROS in G6PD deficiency-induced cellular senescence.

G6PD and cell death

Apart from its role in growth and senescence, G6PD may play an important role in death signaling. Human fibroblasts deficient in G6PD activity showed an altered biological response to nitric oxide (NO) (104).

Deficient cells underwent apoptosis after treatment with NO donor. This is in contrast to normal cells in which proliferation was enhanced by the same treatment. Very likely, the cellular G6PD status modifies the signaling pathway in such a way that switches the outcome of cells from life to death.

G6PD and development

As mentioned earlier, G6PD deficiency seems to be incompatible with mammalian development. This issue has been studied by creation of G6PD knockout mice. The earliest attempt by Pandolfi *et al.* Resulted in production of mouse embryonic stem (ES) cells with an inactivated G6PD gene (46). These cells were viable, but they showed a very high sensitivity to oxidative stress, suggesting that G6PD is dispensable for pentose synthesis but essential for antioxidant defense. In a similar fashion, Filosa *et al.* used a crelox approach to generate a G6PD-nullizygous ES line, which was unable to cope with exogenous oxidants (105). Their study is particularly interesting in the following aspect: expression of the bacterial form of G6PD could substitute for the endogenous enzyme in protection against oxidative stress. Under conditions that encouraged in vitro differentiation, deficient ES cells were able to give rise to mesodermal cells, cardiomyocytes, hepatocytes, and primitive erythroid cells in embryoid bodies. However, definitive erythocytes underwent apoptosis after hemoglobin switching (106).

It is reasoned that adult hemoglobin with a lower oxygen affinity readily releases oxygen, leading to excessive oxidative damage and death in G6PD-deficient erythroid cells. These studies emphasized that G6PD serves primarily in maintenance of the proper redox balance. A murine model of a "nearly-complete" G6PD deficiency was generated (107). ES cells used in this study still retained about 2-30% of normal G6PD activity as a result of an artificial splicing. The mouse generated from this ES line displayed an intriguing inheritance pattern: after crossing the chimera with a normal female, the first generation (F1) G6PD (+/-) heterozygotes born were phenotypically normal. However, when F1 female heterozygotes were bred to normal males, only normal mice were born. Hemizygous G6PD (-) males as well as heterozygous G6PD (+/-) females died *in utero*. The latter were supposedly devoid of G6PD activity as a consequence of inactivation of the paternal X chromosome. It was found that the placental development of these embryos was severely impaired. Longo *et al*. (107) hypothesized that these embryos may die from oxidative stress and damage upon the establishment of circulation and impairment of placental function.

G6PD and Viral Infection

G6PD-deficient cells provide a good model for studying how an altered redox balance affects cellular physiology. One area that has aroused much interest is the interaction between oxidative stress and viruses.

It is well-documented that the redox environment affects the outcome of viral infection. For example, replication of Coxsackievirus, rhinovirus and influenza virus is modulated by redox milieu (108, 109); Coxsackieviruses replicated to a higher titer in C3H/JHe mice fed with diets deficient in selenium (Se), vitamin E or both than in mice given a normal diet (110); glutathione administration has an antiviral effect on influenza virus (111). All these findings suggest that redox imbalance is conducive to viral replication and virulence. Preliminary findings have shown that the cellular G6PD status determines the outcome of enteroviral and coronaviral infection. It appears that G6PD deficiency enhances both the cytopathic effect and the number of progeny viruses produced.

Problems to be solved

With the advancements in the field of G6PD research in the past few years, it has become certain that G6PD is an indispensable component of antioxidant defense. Still, whether G6PD deficiency plays pathogenic roles in diseases other than hemolytic disorders remains to be clearly defined.

G6PD and cancer

The correlation between G6PD and cancer is ambiguous. Previous studies revealed elevated G6PD activities in malignant tissues in various cancers (112). Consistent with this, NIH3T3 cells overexpressing G6PD gave rise to tumors in nude mice, implying that G6PD is a promoter of tumorigenesis (113). On the other hand, several epidemiologic studies did not reveal any difference between G6PD-deficient and healthy patients (114, 115). A more recent mortality follow up study in Sardinia showed an association between G6PD deficiency and non-Hodgkin's lymphomas (116). More epidemiological studies are needed to make definite conclusions. Perhaps, G6PD- deficient individuals may be less prone to the risk of cancers because of diminished proliferative potential and premature senescence of their cells. Additional mutations in key regulatory genes like p53 may be needed for these cells to evade senescence. This also means that the role of G6PD deficiency will be overshadowed by mutations of these genes. Moreover, G6PD deficiency may affect tumorigenesis indirectly. Indeed, we have found an association between G6PD activity and the relapse rate in nasopharyngeal cancer (117).The mechanism behind this is currently unknown.

G6PD and common disorders

It appears that G6PD deficiency is associated with an increased susceptibility to certain diseases. For instance, G6PD-deficient individuals suffer from an increased risk of diabetes and cataract (118). It is plausible that the reduced proliferative capacity of G6PD-deficient cells impairs the turnover of damaged parenchyma cells. This, together with increased oxidative damage, can undermine the normal physiological functions of various tissues. G6PD may play subtle roles in other aspects of health. For example, G6PD deficiency predisposes affected subjects to a higher risk of hypertension. This may be due in part to impaired production of nitric oxide (118, 119).

Conflict of interests

The authors declare that they have no competing interests.

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