Metabolic Reprogramming in Sickle Cell Diseases

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Sickle cell disease (SCD) is a genetic disorder that affects millions of individuals worldwide. Chronic anemia, hemolysis, and vasculopathy are associated with SCD, and their role has been well characterized. These symptoms stem from hemoglobin (Hb) polymerization, which is the primary event in the molecular pathogenesis of SCD and contributes to erythrocyte or red blood cell (RBC) sickling, stiffness, and vaso-occlusion. The disease is caused by a mutation at the sixth position of the β-globin gene, coding for sickle Hb (HbS) instead of normal adult Hb (HbA), which under hypoxic conditions polymerizes into rigid fibers to distort the shapes of the RBCs. Only a few therapies are available, with the universal effectiveness of recently approved therapies still being monitored.

Keywords: sickle cell disease ; glycolysis

1. Introduction

Sickle cell disease (SCD) is a genetic disorder that affects millions of people worldwide [1][2]. The pathophysiology of the disease is directly associated with a pathogenic mutation in the oxygen (O_2) transport protein, hemoglobin (Hb) [2][3]. The primary function of Hb is to transport oxygen from the lungs to the tissues, binding and releasing the oxygen cooperatively, the latter with the help of the natural allosteric effector, 2,3-bisphosphoglycerate (2,3-BPG). During that process, Hb equilibrates between two conformational states: (1) the high O_2-affinity relaxed (R) Hb, or oxygenated Hb, and (2) the low O_2-affinity tense (T) Hb, or deoxygenated Hb (deoxyHb) [2][4]. Normal human adult Hb (HbA) is a tetrameric protein consisting of two α-subunits (α_1 and α_2) and two β-subunits (β_1 and β_2) in a tetrahedral arrangement with a large central water cavity. Each subunit has a prosthetic heme group that binds ligands, including oxygen. Several naturally occurring Hb variants have been implicated in SCD pathologies [4][5][6][7][8][9][10], with the most well-known variant sickle Hb (HbS), resulting from a single-nucleotide mutation in the β-globin gene that codes for βVal6 instead of βGlu6 [7][8]. Unlike HbA, HbS tends to polymerize or aggregate under hypoxia or when deoxygenated, because of an interaction between the pathological β_2Val6 from one deoxyHbS molecule and a hydrophobic acceptor pocket in a proximate deoxyHbS molecule containing the amino acids β_1Ala70, β_1Phe85, and β_1Leu88 [2][3][8]. The initial polymer formation becomes pathological only when stabilized by several secondary contacts between the HbS molecules. Consistently, mutations that break some of these secondary contacts, for example, αAsn78→Lys (Hb Stanleyville), βAsp73→Asn (Hb Korle Bu), or βAsp73→Val (Hb Mobile), increase the solubility of deoxyHbS, thereby reducing the severity of the disease [2][4][8][9][10]. Interestingly, 2,3-BPG, which is found in high concentration in sickle erythrocytes or red blood cells (RBCs), in conjunction with the hypoxia-responsive signaling molecule, sphingosine 1-phosphate (S1P) [11], adversely decreases HbS affinity for oxygen, leading to an increased concentration of the polymer-forming low-O_2-affinity deoxyHbS [12][13][14][15][16][17][18]. Polymerization results in the formation of a helical structure, which ultimately leads to the sickling of RBCs and impaired rheology of the blood [2][3][7][8][10]. Adhesion between the sickled RBCs, neutrophils, endothelium, and platelets occurs, leading to vaso-occlusion (VOC) that eventually results in complications, such as ischemia, painful VOC crises, and acute chest syndrome [2][3][19][20][21]. Sickle RBCs are also susceptible to oxidative damage and hemolysis, resulting in hemolytic anemia [2][3][19][20][21]. Other complications of the disease include stroke, bone infarcts and necrosis of the femoral head, leg ulcers, splenic infarction, and pulmonary hypertension [2][3][19][20][21].

2. Glucose Metabolism

Red blood cells are primarily responsible for transporting oxygen from the lungs to the tissues and returning carbon dioxide from the tissues to the lungs as a byproduct; both processes require energy to operate properly [22]. As RBCs lack intracellular organelles, their primary source of energy is via anaerobic glycolysis [22][23][24]. Glucose molecules are broken by two important pathways—glycolysis, also referred to as the EMP pathway, and the PPP, also referred to as hexose monophosphate shunt (HMP) [24][25][26][27][28]. The EMP pathway, the main pathway in glucose metabolism, breaks down glucose into pyruvate or lactate, producing adenosine triphosphate (ATP) as a source of energy to accomplish cellular processes [24][25][28]. It also facilitates the production of the reducing agent, nicotinamide adenine dinucleotide (NADH), as

well as 2,3-BPG to regulate the oxygen carrying capacity of Hb [24][25][28]. The HMP shunt, however, is an alternate pathway in which glucose is broken down into different metabolic intermediates required for cellular protection against oxidative damage [22][24][25][26][28]. The flux of glucose through both pathways depends on O_2 variation in the cells [22][24][25][26]. When RBCs are oxygenated, glucose metabolism via EMP is inhibited, while metabolism through PPP is induced to combat the oxidant stress [22][24][25][26][28]. In contrast, when RBCs are deoxygenated, glucose metabolism through the EMP pathway is induced to compensate for the hypoxia [22][24][25][26][28]. The activities of the glycolytic enzymes are crucial to maintain RBC homeostasis, and dysfunction of any of these enzymes or the redox system can lead to several hematological disorders and/or their underlying pathophysiologies [29].

3. Glucose Metabolism and Pathophysiology of SCD

3.1. SCD and the Glucose Flux Switch toward Glycolysis Relative to the Pentose Phosphate Pathway

From the forgoing, it is evident that the balance between the activities of the EMP and PPP pathways is crucial for the normal physiological function of erythrocytes. Metabolic reprogramming in sickle RBCs, where PPP glucose flux is switched toward glycolysis, is expected to cause an increase in 2,3-BPG production while decreasing the production of NADPH, and, subsequently, a decrease in the concentration of the antioxidant GSH [12][13][30][31][32][33]. This metabolic switch will impact the ability of sickle RBCs to detoxify the reactive oxygen species, leading to a cascade of events that ultimately worsens the symptoms of SCD [12][13][32][33][34].

Thus, targeting the PPP pathway, especially to increase the production of NADPH, may serve to decrease oxidative stress in sickle RBCs and provide a therapeutic option for SCD. Interestingly, one of the recently approved drugs, Endari (L-glutamine) works by reducing oxidative stress and the associated complications of the disease [35]. During the oxidative phase of the PPP pathway, two NADPH are produced by two-step catalyzed reactions by the enzymes glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) (**Figure 2**) [36]. These enzymes are, therefore, potential targets for increasing the production of NADPH to counter the serious pathological problem of oxidative stress in sickle RBCs.

3.2. The Role of 2,3-BPG in SCD Pathophysiology

The oxygen carrying function of Hb is closely associated with 2,3-BPG. Under normal physiological conditions, only about 25–40% of the oxygen bound to Hb is released to tissue, which is made possible by the preferential binding of 2,3-BPG at the β-cleft of deoxyHb to decrease the protein affinity for the bound oxygen [4][37]. In sickle RBCs, the concentration of 2,3-BPG is significantly elevated, which allows rapid and increased release of oxygen even before the blood reaches the tissue beds [14]. This adaptive response is required to counter the chronic anemia due to the loss of Hb from constant hemolysis by the brittle sickle RBCs [4][14][38][39]. Nevertheless, this response is counterproductive since it leads to an increased concentration of the polymer-forming deoxyHbS. Evidence also suggests that 2,3-BPG is involved in direct stabilization of the HbS polymers [40]. Unlike individuals carrying the homozygous sickle cell gene (HbSS), who suffer from severe illness, individuals with sickle cell trait (HbAS) usually exhibit no significant clinical symptoms. Interestingly, HbAS individuals with inherited PK deficiency have the same severe clinical phenotypes as HbSS individuals due to high elevation of 2,3-BPG concentration in the RBCs, further supporting the importance of 2,3-BPG in disease pathogenesis [41][42]. Expectedly, decreasing 2,3-BPG levels in sickle RBCs has been shown to reduce HbS polymerization and RBC sickling [16][17].

3.3. The Combinatorial Role of 2,3-BPG and S1P in SCD Pathophysiology

Sphingosine-1-phosphate (S1P) is a signaling molecule involved in regulating many cellular processes, such as angiogenesis, cell proliferation, migration, endothelial injury, and inflammation [11]. S1P has been shown to be elevated in the blood of humans and mice with SCD due to the increased activity of sphingosine kinase 1 (SphK1) promoting sickling, hemolysis, inflammation, and multiple tissue damage [12][13]. Sphk1 knockdown in SCD mice significantly reduced sickling due to lowering S1P levels in erythrocytes [12]. Moreover, genetic deletion of Sphk1 in SCD mice was also observed to significantly lower 2,3-BPG production [12]. Interestingly, 2,3-BPG and S1P work together synergistically to decrease Hb affinity for oxygen, promoting deoxygenation and contributing to erythrocyte sickling [12][13]. Under normal O_2 tension, the main glycolytic enzymes, such as GAPDH, aldolase, phosphofructokinase, pyruvate kinase, and lactate dehydrogenase form a complex with the RBCs membrane protein Band 3 (cdB3), rendering the enzymes inactive [12][13]. However, in low O_2 tension, deoxyHb binds to cdB3 (mediated by S1P and 2,3-BPG), to displace and release the glycolytic enzymes from cdB3 into the cytosol [12][13]. This leads to activation of glycolysis, which promotes glucose to enter the glycolytic pathway rather than the PPP, ultimately leading to a suppression of glutathione production and a subsequent increase in oxidative stress [12][13]. The enhanced glycolysis also increases production of 2,3-BPG, resulting in increased formation of the

polymer-forming deoxyHbS, and the associated HbS polymerization and RBC sickling [12][13]. Interestingly, S1P only binds to deoxyHb in the presence of 2,3-BPG, which again serves to highlight the crucial role of 2,3-BPG in SCD pathogenesis [12][13].

4. Drug Discovery Opportunities and Challenges

4.1. Glycolytic Enzymes

4.1.1. Erythrocyte Pyruvate Kinase (PKR)

PK has four different isoenzymes in human tissue: (1) PKL, which is mainly found in the liver; (2) PKR, which is found in the RBCs; (3) PKM1, which is found in the muscles, heart, and brain; and (4) PKM2, which is found in early fetal tissue [43][44]. PKL and PKR isoenzymes are expressed from the gene *PKLR*, while PKM1 and PKM2 are expressed from the gene *PKM*.

PKR-deficient reticulocytes, caused by a mutation in the PKLR gene, have been shown to have a reduced lifespan through selective destruction in the spleen [45]. Additionally, ATP depletion in PKR-deficient reticulocytes cells leads to increased RBC dehydration and destruction [46][47], causing chronic nonspherocytic hemolytic anemia (CNSHA) [48]. For this reason, PKR activators have potential application for the treatment of hemolytic anemia caused by pyruvate kinase deficiency. PKR deficiency is also associated with reduced Hb oxygen affinity as a result of increased 2,3-BPG production, which leads to an increased concentration of the polymer-forming deoxyHbS [42]. The involvement of PKR activity in the regulation of ATP and 2,3-BPG makes it a potential target for SCD therapeutics. In fact, two PKR activators, AG-348 and FT-4202, are currently in clinical trials for the treatment of SCD [49][50].

4.1.2. Bisphosphoglycerate Mutase (BPGM)

BPGM is a homodimer, with each monomer composed of two domains that are formed by six β-strands (named βA-F) and ten α-helices (named α1-10) with a molecular weight of 30 kDa per monomer [51][52]. The dimer is formed between the surface of the βC strands and α3 helices of the two monomers (PDB: 7n3r). Interestingly, the three distinct reactions of BPGM are catalyzed at the same active site and share the same substrates and cofactors, imposing difficulties in assaying for 2,3-BPG production or BPGM activity. The phosphatase activity of BPGM has been reported to be activated by different effectors, such as chloride, sulfite, inorganic phosphate, and, most potently, 2-phosphoglycolate (2-PG) [53][54]. 2-PG is a physiological activator that exists in RBCs at a concentration of 2–5 μM [53]. A study published by Poillon et al. showed that activation of the phosphatase activity in the presence of glycolate resulted in a decrease of its 2,3-BPG level, consequently improving the solubility and ameliorating the sickling tendency of sickled RBCs [16][17]. Furthermore, a study by Knee et al. evaluated the effect of 2,3-BPG elimination with respect to the SCD pathology through a complete knockout of the BPGM gene in Townes model mice [55]. The BPGM-knockout mice had an increased Hb affinity for oxygen with a 59% reduction in RBC sickling [55]. While there are no known synthetic modulators of BPGM, its uniqueness to erythrocytes and its central role in 2,3-BPG production positions BPGM as an ideal target for SCD therapeutics.

4.1.3. Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH)

GAPDH functions by mediating O_2-dependent metabolic variation through its facilitation of the binding of metabolic enzymes and deoxyHb to the N-terminal cytosolic domain of band 3 [12]. Normally, GAPDH is inactivated when bound to band 3 but becomes activated once it is released to the cytosol [12]. Under the RBC sickling condition, deoxyHbS forms a ternary complex with 2,3-BPG and S1P and binds with a high affinity to band 3, thereby displacing GAPDH into the cytosol, leading to an increased activity and consequent metabolic shift from PPP to glycolysis [12]. Unfortunately, the increase in glycolysis results in a detrimental cycle in which 2,3-BPG levels are elevated, leading to an increased concentration of deoxyHbS and, thus, more RBC sickling. In turn, this causes a decrease in antioxidant production, making the cells unable to detoxify the reactive oxygen species and, ultimately, leading to RBC hemolysis and oxidative stress. It is worth noting that GAPDH is naturally inhibited by S-glutathionylation, which is severely reduced in SCD [56][57]. Therefore, the inhibition of GAPDH activity could potentially interrupt this cycle, as a therapeutic strategy to ameliorate SCD.

4.1.4. Triosephosphate Isomerase (TPI)

GAP is central to both glycolysis and PPP, and it has been postulated that the PPP shunt works at near-maximal levels during TPI deficiency because PPP is linked to glycolysis through the intermediates G6P and GAP [58]. DHAP, alternatively, is a dead-end product in erythrocytes but is shunted into lipid metabolism in the brain [59]. DHAP is also known to decompose nonenzymatically to produce methylglyoxal that can lead to formation of glycation products (AGEs) [59][60]. Impairment or inactivation of TPI results in increased concentrations of PPP metabolites, as glucose is redirected

to the PPP, thus increasing the formation of NADPH which helps to protect the cell from oxidative stress [61]. In fact, PPP activation has been proposed as a compensatory strategy for lower TPI activity [59]. Consistently, individuals with TPI deficiency showed an activation of G6PD, which catalyzes the rate-limiting phase of the PPP, further supporting this theory [59]. As such, TPI inhibitors could potentially be explored as possible SCD therapeutic agents.

4.2. PPP Enzymes

Glucose-6-phosphate Dehydrogenase (G6PD)

The main role of NADPH, which is a cofactor of G6PD, is to protect the cell from oxidative damage. Deficiency of G6PD, therefore, perturbs NADPH homeostasis, thereby impairing the ability of the cell to detoxify free radicals, leading to several pathological problems, such as hemolytic anemia [62]. G6PD deficiency has been linked to increased severity of anemia in SCD patients [63][64]. This, together with the fact that G6PD is the rate limiting step of PPP, suggests that activation of G6PD could be a potential strategy for managing SCD. Structurally, G6PD equilibrates between a dimer and a tetramer with a molecular weight of 59 kDa per monomer [65][66][67]. Each monomer consists of two co-enzyme ($NADP^+$)-binding domains and a G6P-binding site that is located between these two domains. Recently, a small-molecule activator of G6PD, AG1, was discovered [66] and was suggested to bridge the dimer interface at the $NADP^+$-binding sites of the two interacting G6PD monomers to induce a conformational change that activates its enzymatic function. While AG1 has not been tested for its effect on SCD, it was shown to reduce oxidative stress in zebrafish, which further supports the potential of G6PD activators as a class of future SCD therapeutics [66][67].

5. Conclusions

Sickle cell disease is an inherited chronic blood disorder that presents at birth. SCD is characterized by polymerization of deoxyHbS and concomitant sickling of RBCs [2][3][7][8][10]. Mature RBCs are responsible for transporting oxygen from the lung to tissues and they procure their energy via anaerobic glycolysis, which is required to maintain the structural integrity of the RBCs [4][23][24]. Two essential mechanisms break down glucose molecules: glycolysis or the EMP pathway, and the PPP or HMP pathway [24][25][26]. The flow of glucose across both channels is influenced by the amount of oxygen in the cells. Glucose metabolism via EMP slows when RBCs are oxygenated but speeds up via PPP to resist oxidant stress. When RBCs are deoxygenated, glucose is mostly metabolized via the EMP route to compensate for the hypoxia. Thus, maintaining RBC homeostasis requires the activity of several glycolytic enzymes. Hematological disorders and/or their associated pathophysiologies can be caused by a malfunction of any of these glycolytic enzymes and/or the redox system [24][25][29][30][31][32][68][69][70]. Theoretically, inhibition of GAPDH and TPI could also help in the treatment of SCD. There are, however, several challenges posed by targeting the glucose metabolic pathway for drug discovery. Flux through the metabolic pathways is modulated through a combination of regulation of enzyme activity by small molecules, as well as regulation of protein levels by hormonal control of tissue-specific gene expression. Thus, targeting a specific enzyme, either totally inhibiting it or allosterically regulating it, could lead to modulation of the whole metabolic pathway as an adaptation mechanism, resulting in unintended consequences.

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