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Homeostasis of Oxidative-Related Metabolism in Human Erythrocyte: An Overview of the Hemoglobin S Presence Implications

Nayara Alves Chaves[1] and Danilo Grünig Humberto da Silva[2]*

[1]*Department of Biology, São Paulo State University (UNESP), Brazil*

[2]*Department of Chemistry and Enviromental Sciences, São Paulo State University (UNESP), Brazil*

***Corresponding author:** Danilo Grünig Humberto da Silva, Department of Chemistry and Environmental, Sciences, São Paulo State University, Cristovão Colombo Street 2265, Jardim Nazareth, Brazil

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Abstract

Sickle erythrocytes are characterized by the presence of sickle hemoglobin (HbS), instead of normal one (Hb). HbS suffers recurrent polymerization/de polymerization, it has tendency to autoxidise faster than Hb, leading to formation of hemi-chromes, and increased and unremitting pro oxidant generation. As consequence, sickle erythrocytes show a hyper-oxidative state that appears to be involved in the rupture of the metabolic homeostasis in this cell. This rupture causes a series of clinical complications in a vicious cycle in which many different oxidative processes play a central role in the patho physiology of sickle cell diseases. Thus, in this review, we briefly overview the implications of the HbS presence in the disruption of erythrocyte metabolic homeostasis, addressing the integration of some erythrocyte metabolic pathways, such as redox, glycolytic, pentose phosphate, methemoglobin reductive and Rapoport-Luebering ones.

Key words: Hemoglobin S; Oxidative processes; Antioxidants; Metabolic integration

Introduction

Sickle Cell Disease (SCD) is one of the most important hemoglobinopathies and is the most common severe monogenic disorders worldwide [1]. The term SCD embraces a group of genetic conditions in which pathology results by substitution of the 20th nucleotide, adenine by thymine, resulting in the exchange of glutamic acid (hydrophilic characteristics) by valine (hydrophobic characteristics), in the sixth position of the chain (HBBglu6val) [1-3]. Thus, results in different structural and biochemical characteristics of normal hemoglobin (Hb) and formation of hemoglobin S (HbS) [4,5]. This amino acid exchange favours interactions between hydrophobic residues of the protein under conditions of low oxygen tension, dehydration or acidosis, forming polymers [5]. These grow, break the cytoskeleton and fill the erythrocyte, causing modifications in its architecture and flexibility, culminating in the formation of sickle erythrocytes, which are less flexible, more adherent to the endothelium and prone to hemolysis [6,7].

The clinical complications in SCD include hemolytic anemia, endothelial dysfunction, inflammation, and hypercoagulability, effects of ischemia-reperfusion injury, hypoxemia and damage to multiple organs [7-10]. These complications have a cyclic nature and the oxidative stress process acts as cause and consequence [11]. In addition to the relationship with patho-physiological events that may imply in more severe clinical outcomes, the hyper-oxidative state of the sickle erythrocyte appears to be involved in the rupture of the metabolic homeostasis in this cell [7]. Once it has been demonstrated alterations in the erythrocyte redox metabolism [11], as well as in the activities of the enzymes of the glycolytic and the phosphate pentose pathways, resulting in function impairments of these pathways [12,13], and altered levels of pyridine nucleotides and purine metabolites [13].

Many studies have elucidated oxidative stress in SCD or in red blood cell (RBCs). On the other hand, to the authors' knowledge, there are no works addressing the implications of the HbS presence in the disruption of erythrocyte metabolic homeostasis, regarding oxidative-related processes. Thus, in this review, we briefly overview the integration of some erythrocyte metabolic pathways, such as redox, glycolytic, pentose phosphate, methemoglobin reductive and Rapoport-Luebering ones.

Redox–Related metabolism of Human HbA-Containing Erythrocyte

RBCs are highly specialized and the most abundant cells in the human organism [14,15]. Their primary function is oxygen (O2) transportation from the lungs to the tissues [14,15]. Thus, these cells are highly susceptible to oxidative damage, due to the high concentration of O2 and Hb, which undergoes auto-oxidation,

producing methemoglobin (MetHb) and superoxide radical (O2•-) that is an important trigger of the oxidative processes [16-18]. Although it is a normal physiological process, even a small rate of Hb autoxidation can produce substantial oxidant specie levels, since more than 95% of erythrocyte cytoplasm protein content is composed of Hb, whose concentration bound to O2 is approximately 5mM [19].

In addition to dealing with an internal environment in constant oxidant specie production, during human RBC lifespan of about 120 days, erythrocytes are exposed to a large number of stressful oxidative situations [17,20]. For instance, RBCs pass through the lungs at least one time per minute, where they face a highly pro-oxidative environment [21]. Furthermore, human erythrocytes are exposed to oxidants produced in the circulation [22], as well as to a wide range of oxidative xenobiotics. Thereby, the integrity of erythrocyte redox metabolism is constantly challenged, as well as the related metabolic pathways.

In this scenario, in order to prevent or attenuate the oxidative stress generated in the cell, RBCs are equipped with an effective and self-sustaining antioxidant system that makes them mobile free radical scavengers, providing antioxidant protection, both enzymatic and non-enzymatic [11]. Activity of antioxidant defence enzymes, including superoxide dismutase (SOD), which enables the radical O_2•- from the auto-oxidation of Hb to be disputed into O_2 and hydrogen peroxide (H_2O_2), posterior, catalase (CAT) neutralizes the H_2O_2, transforming it in H2O and O_2 [23]. Other enzymes that contribute to the reduction of lipid/alkyl peroxides as well as other organic hydroperoxides are glutathione per-oxidise (GPx) and per-oxiredoxins (Prx), using reduced glutathione (GSH) as cofactor [23,24]. GSH also acts as cofactor for glutathione-S-transferees (GST) in the detoxification of xenobiotics and of glutaredoxin (Grx), responsible for the reduction of oxidized proteins and as corbate [23]. In addition, GSH protects important membrane proteins against oxidation, such as spectrin, favouring the maintenance of the integrity and flexibility of the erythrocyte membrane [25], and it is an integral part of the sulfhydryl group pool that keeps Hb in its reduced state [26].

Under conditions associated with excessive oxidant generation, high levels of oxidized GSH obtained, e.g. glutathione disulfide (GSSG) can be externalized in order to prevent cytotoxicity. This mechanism may be responsible for the decrease of GSH levels or decreased GSH/GSSG ratio in erythrocytes [27,28]. However, GSSG can be reverted to its reduced form by the action of glutathione reductase (GR), which uses NADPH, as a reducing agent [27,28]. Likewise, thioredox in reductase (TR) uses NADPH from the pentose phosphate pathway (PPP) to reduce oxidized thioredoxin (TrxS2) to its dithiol form (Trx $(SH)_2$), the latter being responsible for reducing PrxS2 [29,30]. Undoubtedly the per-oxidase activity of Prx enzymes, a very large and highly conserved family of peroxidases, is critical to protect RBCs from oxidative damage [31], since Prxs are the third most abundant protein in the erythrocyte [29,31]. In addition to their peroxidase function, Prx2 (one of the six isoforms found in humans) appears to act as Hb molecular chaperone under conditions of oxidative stress [32]. For instance, during erythropoiesis by maintaining adequate Hb folding and after erythrocyte maturation, by preventing Hb denaturation [29,33,34]. Furthermore, Prx2 binds to free heme with high affinity, probably in order to avoid oxidative reactions triggered by it [35].

Another constituent of the intra-erythrocyte sulfhydryl group pool is ergothioneine (Ergo), which, curiously, is the second most abundant thiol in RBCs [26]. Thus, it seems logical to assume that Ergo is an antioxidant specialized in the protection of this cell type, but the reasons for such abundance have not yet been sufficiently investigated. Furthermore, RBCs have a plasma membrane redox system (PMRS) that transfers electrons from intracellular substrates to extracellular electron acceptors which may be NAD+ or/and dehydroascorbic acid [36]. Therefore, RBCs uniquely function to protect Hb via a selective barrier, allowing gaseous and other lig and transport as well as providing enzymatic mechanisms to maintain Hb in a functional nontoxic state [37], and antioxidant protection not only to themselves but also to other tissues and organs in the body [38].

In addition to the antioxidant system, erythrocytes have an efficient energy production, which derives exclusively from glucose degradation through the glycolytic pathway and subsequent lactate production, due to the absence of mitochondria, producing ATP and NADH [39,40], while ATP is involved in the maintenance of erythrocyte integrity, NADH is the main reducing agent of met Hb, keeping Hb in the ferrous state. This last reaction is catalyzed by NADH-cytochrome b5 reductase, also known as the meta Hb reductase [41]. Human erythrocytes express very high levels of insulin-independent glucose transporter (GLUT1) that ensures, through passive transport, high glucose concentration in the erythrocyte cytosol, normally close to that in the plasma [42,43]. Approximately 90% of that glucose is metabolized through the glycolytic pathway to produce ATP [23]. Moreover, RBCs have a nucleotide metabolism that assists in the maintenance of the energy balance in erythrocytes, through the purine metabolic cycle [44]. The remaining glucose is directed to the PPP, whose main function is the production of redox potential in the form of NADPH [40], by successive oxidation reactions catalyzed by glucose-6-phosphate dehydrogenase (G6PDH) and phosphor gluconate dehydrogenase [23,40].

The close relationship between the glycolytic and PPP is evident, once the glycolytic flux is modulated by a competition between glycolytic enzyme, phosphofructokinase-1 (PFK-1) and deoxygenated Hb (deoxy-Hb) by the cytoplasm domain of the protein band 3 (CDB3) [45]. Briefly, conditions of low concentration of O2 promote the release of PFK-1 from CDB3, induced by deoxy-Hb binding, increasing the flow of glucose to glycolytic, consequently the production of 2, 3-BPG, through Luebering - Rapoport pathway, which allosterically regulates O_2 release from Hb [46]. On the other hand, since glucose consumption is constant, in erythrocytes exposed to high O_2 concentrations,

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PFK-1 is inhibited by its binding to CDB3, favouring glucose directing to PPP, in order to ensure adequate levels of NADPH needed to protect the erythrocytes of lesions triggered by oxidant [45]. Furthermore, Zhang et al. [43] demonstrated in vitro that under induced oxidative stress situations, erythrocytes shifted glucose metabolism towards the oxidative PPP, seeking NADPH production for oxidant mitigation. However, perturbations in these pathways involved in RBC cellular function and survival can lead to an enhanced flow of pro-oxidant generation, culminating in oxidative stress, consequently in loss of metabolic homeostasis and premature senescence.

Disruption of Redox-Related Metabolism by Hbs

Sickle erythrocytes are characterized by the presence of HbS, instead of normal HbA. As mentioned previously, HbA undergoes autoxidation at normal physiological rate, while HbS is highly unstable thus altering this rate. According to Hebbel et al. [47], HbS has tendency to autoxidise 1.7 times faster than HbA. This accelerated autoxidation causes premature HbS denaturation, leading to formation of hemi chromes, which have high affinity for CDB3 [48,49], mediating the oxidative cross-linking of CDB3 by disulfide bonds [50]. The result is the band-3 clustering and its dissociation of cytoskeleton proteins by ankyrin binding rupture [50,51]. As mentioned before, one of the band-3 functions is to bind to glycolytic enzymes and organize them into the membrane, thereby regulating the glucose flux between glycolysis and PPP [52]. Therefore, the release of glycolytic enzymes from the oxidized band-3 might be responsible for the alterations in the activities of glycolytic and PPP enzymes [12], as well as in the levels of NADH and NADPH [53], previously reported in the literature, in sickle erythrocytes.

Moreover, upon de-oxygenation, HbS molecules expose hydrophobic contacts formed between valine of one HbS molecule and alanine, phenylalanine and leucine from an adjacent HbS [54,55]. This crystallization produces a polymer nucleus, which grows and fills the erythrocyte, disrupting its architecture and flexibility and promoting cellular dehydration, with physical and oxidative cellular stress [56]. Nevertheless, HbS polymerization is reversible; fibers "melt" as oxygen is taken up by the HbS and the normal discoid shape returns [10]. This re-oxygenation phase might be considered the major source of pro-oxidant production in SCD [18,57]. Hence, sickle erythrocytes have been reported to generate two fold greater extent of O2•−, H2O2, hydroxyl radical (HO•) and lipid oxidation products compared with HbA-containing erythrocytes [18,47,58]. The increased and unremitting pro-oxidant generation in sickle erythrocytes results in excessive antioxidant consumption and thus antioxidants deficiency [11,59,60] that may also trigger glucose metabolism shifting.

The repeated polymerization/de-polymerization process can also lead to blood cell adhesion, vaso occlusion, ischemia-reperfusion injury and hemolysis [5,10]. During periods of cellular hypoxia or stress, adenosine is released from cells along with the adenine nucleotides, ATP, ADP, and AMP, which are converted to adenosine by ectonucleotidases [61,62]. In addition, Zhang et al. [63] demonstrated that adenosine can enhance 2,3-BPG production via A2B receptor activation, suggesting that elevated adenosine had an unrecognized role in normal RBCs to promote O_2 release and prevent acute ischemic tissue injury. However, this response is even more pronounced in SCD patients due to increased amounts of ATP in the circulation derived from chronic sickle red cell hemolysis and tissue damage from vaso occlusion [64]. Thus, in sickle erythrocytes, the beneficial role of excessive adenosine-mediated 2,3-BPG induction becomes detrimental by promoting deoxygenating, HbS polymerization and subsequent sickling [63].

Hemolysis increases the concentration of free plasma Hb, which in the ferrous (Fe2+) valence state, is readily available to participate in Fenton-based redox reactions [65]. Under normal conditions, this potential source of oxidative stress is minimized by haptoglobin (Hp) [66]. However, in SCD people, plasma Hp levels are low due to chronic hemolysis [67] that overwhelms endogenous plasma Hp levels and other scavenging mechanisms. Moreover, with extracellular Hb the body not only need to deal with the autoxidation reaction but also with the heme that dissociates from met Hb [68].

Heme is a low molecular weight hydrophobic molecule that can be taken up by cell membranes, plasma proteins, and lipids. In the same manner as Hp-Hb scavenging system, plasma hemopexin (Hpx) sequesters heme in an inert, non-toxic form and transports it to the liver for catabolism and excretion, preventing heme's pro-oxidant and pro-inflammatory effects [66,69]. However, once more the characteristic hemolytic condition of SCD persons overwhelms endogenous scavenging capacity of plasma Hpx [67], enabling further deleterious effects of heme and by-products. Taken the above observations into consideration, the presence of HbS generates a series of Consequences for individuals in a vicious cycle in which many different oxidative processes play a central role in the path physiology, thus in the further clinical consequences.

Conclusion

Human erythrocytes are normally exposed to continuous pro-oxidant sources, both intra and extra cellular, as a consequence of their physiological role. Although reductive capacity of an erythrocyte is 250 times higher than its potential oxidizing agents are, HbS is capable of several alterations that overwhelm the antioxidant defence system, leading to the loss of metabolic homeostasis due to the integration of biochemical pathways briefly depicted in this review. Therefore, more studies regarding the main enzymes and by products involved in erythrocyte metabolic homeostasis remains a worthy and promising goal, which might lead to more specific and effective prognostic biomarkers, as well as to new therapeutic targets or agents capable of improve the sickle erythrocyte metabolism.

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Conflict of Interest

The authors declare no competing financial or relationship with other people or organizational interests. Furthermore, the authors have materially participated in the article preparation and approved the final article version.

References

1. Weatherall D, Hofman K, Rodgers G, Ruffin J, Hrynkow S (2005) A case for developing North-South partnerships for research in sickle cell disease. Blood 105(3): 921-923.
2. Serjeant GR (2013) The natural history of sickle cell disease. Cold Spring Harb Perspect Med 3(10): a011783.
3. Steinberg MH, Sebastiani P (2012) Genetic modifiers of sickle cell disease. Am J Hematol 87(8): 795-803.
4. Ngo DA, Steinberg MH (2015) Genomic approaches to identifying targets for treating beta hemoglobinopathies. BMC Med Genomics 8: 44.
5. Rees DC, Williams TN, Gladwin MT (2010) Sickle-cell disease. 376(9757): 2018-2031.
6. Ballas SK, Kesen MR, Goldberg MF, Lutty GA, Dampier C, et al. (2012) Beyond the definitions of the phenotypic complications of sickle cell disease: an update on management. Scientific World Journal 2012: 949535.
7. Barabino GA, Platt MO, Kaul DK (2010) Sickle cell biomechanics. Annu Rev Biomed Eng 12: 345-367.
8. Steinberg MH (2005) Predicting clinical severity in sickle cell anaemia. Br J Haematol 129(4): 465-481.
9. Hebbel RP, Osarogiagbon R, Kaul D (2004) The Endothelial Biology of Sickle Cell Disease: Inflammation and a Chronic Vasculopathy. Microcirculation 11(2): 129-151.
10. Stuart MJ, Nagel RL (2004) Sickle-cell disease. 364(9442): 1343-1360.
11. Silva DG, Belini JE, Almeida EA, Bonini-Domingos CR (2013) Oxidative stress in sickle cell disease: an overview of erythrocyte redox metabolism and current antioxidant therapeutic strategies. Free Radic Biol Med 65: 1101-1109.
12. Lachant NA, Davidson WD, Tanaka KR (1983) Impaired pentose phosphate shunt function in sickle cell disease: a potential mechanism for increased Heinz body formation and membrane lipid peroxidation. Am J Hematol. 15(1): 1-13.
13. Zerez CR, Lachant NA, Lee SJ, Tanaka KR (1988) Decreased erythrocyte nicotinamide adenine dinucleotide redox potential and abnormal pyridine nucleotide content in sickle cell disease. Blood 71(2): 512-515.
14. Foller M, Huber SM, Lang F (2008) Erythrocyte programmed cell death. IUBMB Life 60(10): 661-668.
15. Bhooshan Pandey K, Syed Rizvi I (2011) Biomarkers of oxidative stress in red blood cells. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 155(2): 131-136.
16. Manca L, Masala B (2008) Disorders of the synthesis of human fetal hemoglobin. IUBMB Life 60(2): 94-111.
17. Carrell RW, Winterbourn CC, Rachmilewitz EA (1975) Activated oxygen and haemolysis. Br J Haematol 30(3):259-264.
18. Aslan M, Thornley-Brown D, Freeman BA (2000) Reactive species in sickle cell disease. Ann N Y Acad Sci 899: 375-391.
19. Johnson RM, Goyette G, Jr., Ravindranath Y, Ho YS (2005) Hemoglobin autoxidation and regulation of endogenous H2O2 levels in erythrocytes. Free Radic Biol Med 39(11): 1407-1417.
20. Lang E, Qadri SM, Lang F (2012) Killing me softly - suicidal erythrocyte death. Int J Biochem Cell Biol 44(8): 1236-1243.
21. Lang KS, Lang PA, Bauer C, Duranton C, Wieder T, et al. (2005) Mechanisms of suicidal erythrocyte death. Cell Physiol Biochem 15(5): 195-202.
22. Reiter CD, Wang X, Tanus-Santos JE, Hogg N, Cannon RO, et al. (2002) Cell-free hemoglobin limits nitric oxide bioavailability in sickle-cell disease. Nat Med 8(12): 1383-1389.
23. Van Zwieten R, Verhoeven AJ, Roos D (2014) Inborn defects in the antioxidant systems of human red blood cells. Free Radic Biol Med 67: 377-386.
24. Mannervik B (1987) The enzymes of glutathione metabolism: an overview. Biochem Soc Trans 15(4): 717-718.
25. Carroll J, Raththagala M, Subasinghe W, Baguzis S, D'amico Oblak T, et al. (2006) An altered oxidant defense system in red blood cells affects their ability to release nitric oxide-stimulating ATP. Mol Biosyst 2(6-7): 305-311.
26. Kuypers FA (2014) Hemoglobin s polymerization and red cell membrane changes. Hematol Oncol Clin North Am 28(2): 155-179.
27. Lu SC (2013) Glutathione synthesis. Biochim Biophys Acta 1830(5): 3143-3153.
28. Lu SC (2009) Regulation of glutathione synthesis. Mol Aspects Med 30(1-2): 42-59.
29. Low FM, Hampton MB, Winterbourn CC (2008) Peroxiredoxin 2 and peroxide metabolism in the erythrocyte. Antioxid Redox Signal 10(9): 1621-1630.
30. Lu J, Holmgren A (2014) The thioredoxin antioxidant system. Free Radic Biol Med 66: 75-87.
31. Rhee SG (2016) Overview on Peroxiredoxin. Mol Cells 39(1): 1-5.
32. Moon KH, Kim BJ, Song BJ (2005) Inhibition of mitochondrial aldehyde dehydrogenase by nitric oxide-mediated S-nitrosylation. FEBS Lett 579(27): 6115-6120.
33. Stuhlmeier KM, Kao JJ, Wallbrandt P, Lindberg M, Hammarström B, et al. (2003) Antioxidant protein 2 prevents methemoglobin formation in erythrocyte hemolysates. Eur J Biochem 270(2): 334-341.
34. Poynton RA, Hampton MB (2014) Peroxiredoxins as biomarkers of oxidative stress. Biochim Biophys Acta 1840(2): 906-912.
35. De Franceschi L, Bertoldi M, De Falco L, Santos Franco S, Ronzoni L, et al. (2011) Oxidative stress modulates heme synthesis and induces peroxiredoxin-2 as a novel cytoprotective response in beta-thalassemic erythropoiesis. Haematologica 96(11): 1595-1604.
36. Rizvi SI, Jha R, Maurya PK (2006) Erythrocyte plasma membrane redox system in human aging. Rejuvenation Res 9(4): 470-474.
37. Cappadoro M, Giribaldi G, O'brien E, Turrini F, Mannu F, et al. (1998) Early phagocytosis of glucose-6-phosphate dehydrogenase (G6PD)-deficient erythrocytes parasitized by Plasmodium falciparum may explain malaria protection in G6PD deficiency. Blood 92(7): 2527-2534.
38. Siems WG, Sommerburg O, Grune T (2000) Erythrocyte free radical and

energy metabolism. Clin Nephrol 53(1 Suppl): S9-17.

39. Cavill I (2002) Erythropoiesis and iron. Best Pract Res Clin Haematol 15(2): 399-409.

40. Beutler E (1972) Red cell metabolism. A. Defects not causing hemolytic disease. B. Environmental Modification 54(5): 759-764.

41. Percy MJ, Lappin TR (2008) Recessive congenital methaemoglobinaemia: cytochrome b(5) reductase deficiency. Br J Haematol 141(3): 298-308.

42. Viskupicova J, Blaskovic D, Galiniak S, Soszynski M, Bartosz G, et al. (2015) Effect of high glucose concentrations on human erythrocytes in vitro. Redox Biol 5: 381-387.

43. Zhang ZZ, Lee EE, Sudderth J, Yue Y, Zia A, et al. (2016) Glutathione Depletion, Pentose Phosphate Pathway Activation, and Hemolysis in Erythrocytes Protecting Cancer Cells from Vitamin C-induced Oxidative Stress. J Biol Chem 291(44): 22861-22867.

44. Dudzinska W, Hlynczak AJ, Skotnicka E, Suska M (2006) The purine metabolism of human erythrocytes. Biochemistry (Mosc) 71(5): 467-475.

45. Castagnola M, Messana I, Sanna MT, Giardina B (2010) Oxygen-linked modulation of erythrocyte metabolism: state of the art. Blood Transfus 8 (Suppl 3): s53-58.

46. Rapoport S, Luebering J (1950) The formation of 2,3-diphosphoglycerate in rabbit erythrocytes: the existence of a diphosphoglycerate mutase. J Biol Chem 183(1): 507-516.

47. Hebbel RP, Morgan WT, Eaton JW, Hedlund BE (1988) Accelerated autoxidation and heme loss due to instability of sickle hemoglobin. Proc Natl Acad Sci U S A 85(1): 237-241.

48. Walder JA, Chatterjee R, Steck TL, Low PS, Musso GF, et al. (1984) The interaction of hemoglobin with the cytoplasmic domain of band 3 of the human erythrocyte membrane. J Biol Chem 259(16): 10238-10246.

49. Waugh SM, Willardson BM, Kannan R, Labotka RJ, Low PS (1986) Heinz bodies induce clustering of band 3, glycophorin, and ankyrin in sickle cell erythrocytes. J Clin Invest 78(5): 1155-1160.

50. Mannu F, Arese P, Cappellini MD, Fiorelli G, et al. (1995) Role of hemichrome binding to erythrocyte membrane in the generation of band-3 alterations in beta-thalassemia intermedia erythrocytes. Blood 86(5): 2014-2020.

51. Ferru E, Pantaleo A, Mannu F, Carta F, Cappadoro M, et al. (2010) May band 3 hyper-phosphorylation have a functional role in microcyte formation in heterozygous thalassemias? 45(1): 65-66.

52. Campanella ME, Chu H, Wandersee NJ, Peters LL, Mohandas N, et al. (2008) Characterization of glycolytic enzyme interactions with murine erythrocyte membranes in wild-type and membrane protein knockout mice. Blood 112(9): 3900-3906.

53. Zerez CR, Tanaka KR (1987) Impaired nicotinamide adenine dinucleotide synthesis in pyruvate kinase-deficient human erythrocytes: a mechanism for decreased total NAD content and a possible secondary cause of hemolysis. Blood 69(4): 999-1005.

54. Wishner BC, Ward KB, Lattman EE, Love WE (1975) Crystal structure of sickle-cell deoxyhemoglobin at 5 A resolution. J Mol Biol 98(1): 179-194.

55. Fronticelli C, Gold R (1976) Conformational relevance of the beta6Glu replaced by Val mutation in the beta subunits and in the beta(1-55) and beta(1-30) peptides of hemoglobin S. J Biol Chem 251(16): 4968-4972.

56. Brittenham GM, Schechter AN, Noguchi CT (1985) Hemoglobin S polymerization: primary determinant of the hemolytic and clinical severity of the sickling syndromes. Blood 65(1): 183-189.

57. Hebbel RP (1991) Beyond hemoglobin polymerization: the red blood cell membrane and sickle disease pathophysiology. Blood 77(2): 214-237.

58. Sheng K, Shariff M, Hebbel RP (1998) Comparative oxidation of hemoglobins A and S. Blood 91(9): 3467-3470.

59. Prchal JT, Gregg XT (2005) Red cell enzymes. Hematology Am Soc Hematol Educ Program 19-23.

60. Voskou S, Aslan M, Fanis P, Phylactides M, Kleanthous M (2015) Oxidative stress in beta-thalassaemia and sickle cell disease. Redox Biol 6: 226-239.

61. Jacobson KA, Gao ZG (2006) Adenosine receptors as therapeutic targets. Nat Rev Drug Discov 5(3): 247-264.

62. Eltzschig HK, Carmeliet P (2011) Hypoxia and inflammation. N Engl J Med 364(7): 656-665.

63. Zhang Y, Dai Y, Wen J, Zhang W, et al. (2011) Detrimental effects of adenosine signaling in sickle cell disease. Nat Med 17(1): 79-86.

64. Hasko G, Linden J, Cronstein B, Pacher P (2008) Adenosine receptors: therapeutic aspects for inflammatory and immune diseases. Nat Rev Drug Discov 7(9): 759-770.

65. Belcher JD, Beckman JD, Balla G, Balla J, Vercellotti G (2010) Heme degradation and vascular injury. Antioxid Redox Signal 12(2): 233-248.

66. Kato GJ (2009) Haptoglobin halts hemoglobin's havoc. J Clin Invest 119(8): 2140-2142.

67. Muller-Eberhard U, Javid J, Liem HH, Hanstein A, et al. (1968) Plasma concentrations of hemopexin, haptoglobin and heme in patients with various hemolytic diseases. Blood 32(5): 811-815.

68. Bunn HF, Jandl JH (1968) Exchange of heme among hemoglobins and between hemoglobin and albumin. J Biol Chem 243(3): 465-475.

69. Schaer DJ, Vinchi F, Ingoglia G, Tolosano E, Buehler PW (2014) Haptoglobin, hemopexin, and related defense pathways-basic science, clinical perspectives, and drug development. Front Physiol 5: 415.