Red Blood Cells membrane proteins in patients with diabetes

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Abstract

Red blood cells (RBC) are the major cell component of blood. Their main function is to transport oxygen and carbon dioxide, however, besides the main function of RBCs have also the ability to transport immune complexes, express numerous adhesion molecules, and influence leukocytes and platelets margination and adhesion. The molecular architecture of cell membranes plays a key role in the determination of their function. Modifications of RBC membrane components could alter RBC functions and rheological properties. In this review changes in the protein composition and structure of RBCs membranes in diabetes and their possible diagnostic and prognostic significance are considered.

RBCs membrane proteins are categorized in terms of protein function into three groups: cytoskeletal proteins (spectrin, actin, protein 4.1, etc.), integral structural proteins (band 3, glycophorins (GP), etc.), and anchoring proteins (ankyrin, protein 4.2, etc.). The three principal "cytoskeleton proteins" are spectrin (α and β), actin, and protein 4.1, forming the "junctional complex", that provides support to the lipid bilayer, and maintains the integrity, shape, and architecture of the red blood cell. The unusual properties of RBC cytoskeletal proteins form specialized ensembles, provide a unique combination of flexibility and stability RBCs membrane controlling their biogenesis, survival, and function. The extracellular domain of heavily glycosylated sialoglycoproteins (glycophorins, bend 3 protein) at physiological pH are conferring a negative surface charge to RBCs membrane, which plays a crucial role in modulating RBC–RBC interactions, their interactions with vascular endothelium and the other circulating blood cells.

Tightly association of several RBCs' membrane proteins disorders with different pathological processes was identified. In diabetes, RBC membranes are affected by chronic exposure to glucose, triggering their biochemical modifications (glycosylation, oxidation), with subsequent structural and functional disruption of the cell (disorders of their deformability, adhesion to the endothelium), which is further involved in the pathogenesis of diabetes and its complications. The alterations of RBCs membrane proteins might be useful sensitive biomarkers for long-term glycemic control, early diagnosis, or monitoring of disease progression.

(TCM-GMJ February 2022; 7 (1):P41-P44)

Keywords: Red Blood cells, membrane proteins, defomability, diabetes.

Introduction

Red blood cells (RBC) are the major cell component of blood. Their main function is to transport oxygen and carbon dioxide, however, besides the main function of RBCs have also the ability to transport immune complexes, express numerous adhesion molecules and influence leukocytes and platelets margination and adhesion [Pasini E.M., et al., 2006]. The molecular architecture of cell membranes plays a key role in the determination of their function. The RBC membrane, like most animal membranes, contains 19.5% of water, 39.5% of proteins, 35.1% of lipids, and 5.8% of carbohydrates [Yawata Y., 2003]; proteins and lipids composition and is changing during RBCs lifetime [de Oliveira S., Saldanha C., 2010]. Modifications of RBC membrane components could alter RBC deformability that is most important for their rheological properties. Understanding the relationship between the different components of the RBC membrane helps explain the mechanism of cells' deformability [Deuticke B. 2003; Piagnerelli M., et al., 2003].

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Received June 08, 2020; accepted August 21 , 2020.
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The literature concerning the human RBCs membrane proteins, their localization, and modes of association has been the subject of repeated reviews [Winzler R. J. 1969]. In this review changes in the protein composition and structure of RBCs membranes in diabetes and their possible diagnostic and prognostic significance are considered.

RBCs membrane proteins and their functional role

RBCs membrane proteins are classified into "peripheral proteins" and "integral proteins". The amino acid composition of the erythrocyte membrane protein fraction is not distinctively different from that in other membranes [Steck T.L., 1974], however, individual proteins, and, more particularly, their topographically specified domains have characteristic compositions which relate to their disposition in the membrane. There is a distinct excess of acidic (21%) over basic (12%) amino acid residues in the protein fraction of RBC's membrane. RBCs membrane proteins are categorized in terms of protein function into three groups: cytoskeletal proteins (spectrin, actin, protein 4.1, etc.), integral structural proteins (band 3, glycophorins (GP), etc.), and anchoring proteins (ankyrin, protein 4.2, etc.). Figure 1 shows the topography of the principal polypeptides which comprise about 80% of the RBC's membrane protein mass.

The three principal "cytoskeleton proteins" are spectrin (α and β), actin, and protein 4.1, forming the "junctional complex". Two isoforms of spectrin, alpha (260 kDa) and beta (225 kDa) form a loosely wound helix. Spectrin domains cooperatively fashion, form a dense two-dimensional filamentous network that provides support to the lipid bilayer and maintains the integrity, shape, and architecture of the red blood cell. Spectrin has also been implicated to play important roles in the maintenance of the erythrocyte membrane asymmetry and exhibits chaperon-like activity [Patra M, et al., 2015). Two alpha-beta helixes are linked end to end to form a single tetramer, which has binding sites for several other proteins, and are organized into a meshwork that is fixed to the membrane by the protein ankyrin (215 kDa).

Ankyrin is an anchoring protein that bridges the tight association between spectrin and band 3 [de Oliveira S., Saldanha C., 2010]. The binding can be modulated by the extent of phosphorylation by protein kinase A (PKA), casein kinase I, or cyclic AMP-independent protein.

The short actin microfilaments (their length ~33-37 nm), consisting of 15-18 actin subunits and tropomyosin, are extraordinarily stable, persisting for the lifetime of the RBC. Those are anchored to the membrane at multiple sites. The unusual properties of RBC actin filaments forming specialized ensembles, provide a unique combination of flexibility and stability of cytoskeleton of RBCs membrane; regulation of actin filaments is critical for the membrane skeleton assembly and integrity, thereby controlling the RBC biogenesis, survival, and function.

Band 4.1 (43 kDa) stabilizes the association of spectrin with actin and binds to the transmembrane proteins band 3 and glycophorin C and D [de Oliveira S., Saldanha C., 2010].

Band 3 (90 - 100 kDa) is the most abundant RBC membrane integral protein (composes approximately 25% of proteins) and the major linkage between the cytoskeleton and the lipid bilayer, it can be associated with several proteins of the cytoskeleton. These proteins play a very important role in the regulation of the flexibility and rigidity of RBC. Band 3 is well known to function as an anion exchanger. When red blood cells pass through the lung blood vessels, band 3 protein has a function in collecting CO_2 from human tissues in exchange for Cl^- as the form of $HCO3^-$. Moreover, such an anion exchange function is also involved in pH regulation within cells [Aoki. T., 2017]. Like several other structural proteins, the band 3 configuration can also be modulated by phosphorylation – via phosphotyrosine kinases (PTKs) or dephosphorylation – via phosphotyrosine phosphatase (PTP) [de Oliveira S., Saldanha C., 2010]. In RBCs, hyperphosphorylation of band 3 has been reported in the prooxidant hemolytic disorders, malaria, and intermediate thalassemia, and this phenomenon is closely related to the formation of hemichromes [Pantaleo A. et alo., 2016].

Glycophorins (isoforms of 27 and 29 kD) or sialoglycoprotein are about 2% of total RBC membrane protein. Glycophorins have three domains, (1) a cytoplasmic domain, which contains a cluster of basic residues that are located near the plasma membrane, (2) a hydrophobic domain which exists as a single α-helix spanning the lipid bilayer, and (3) an extracellular domain which is heavily glycosylated [Aoki. T., 2017].

Glycophorin A is the major constituent representing 1.6% of total RBC membrane protein [Yawata Y., 2003]. At physiological pH, sialic acids are negatively charged conferring a negative charge to RBCs. The negative surface charge of RBC plays a crucial role in modulating RBC–RBC interactions and as well RBC interactions with vascular endothelium and the other circulating blood cells [Telen M.J., 2005; Yawata Y., 2003]. RBCs aggregation is frequently associated with vascular occlusion by platelets, or as a preliminary stage in the migration of leukocytes out of the bloodstream and into tissues in the inflammatory process.

Other membrane glycoproteins have low sialic acid content (band 3 protein). Total surface charge density is not affected in GPA-deficient RBCs; these cells exhibit increased glycosylation of band 3, probably due to the addition of excessive sialic acid, which should have been present on the Glycophorin A protein. However, Glycophorins C, D, and band 3 are associated with the cytoskeleton structure and maintenance of the shape and mechanical properties of the red blood cell during passing through capillary vessels [Aoki. T., 2017].

The RBCs membrane represents an example of shear resistant complex imposed on the lipid bilayer by its associated cytoskeletal protein network. The ternary complex of spectrin, actin, and Band 4.1 protein defines the nodes of the RBC membrane skeletal network and is an important component of membrane stability under mechanical stress. The elastic response of the RBC to the loading that it experiences in the microcirculation derives from the network of elongated spectrin (α2β2) tetramers, while the membrane stability results from dimer-dimer interaction of the spectrin and the junctional complex of spectrin-actin-bend 4.1. It was shown that selective depletion of tropomyosin from the membrane results in weakening of the ternary spectrin-actin-4.1 junctional complex and reversible decrease membrane mechanical stability [Xiuli An, et al., 2007].

Alternations of RBCs membrane proteins and their pathophysiological signifiacance

It was observed that several of the RBC disorders are tightly associated with mutations occurring in genes codifying different RBC membrane proteins. For instance, Hereditary Spherocytosis, Hereditary Elliptocytosis, and Southeast Asian Ovalocytotic have associated with mutations in peripheral protein β-spectrin [Aoki. T., 2017]. Band 3 phosphorylation has

been described in intermediate thalassemia [Ferru E., et al., 2014], malaria-infected RBC [Pantaleo A., et al., 2012], and during renal tubular acidosis [de Oliveira S., Saldanha C., 2010]. In Europe, about 30% of the cases of Hereditary Elliptocytosis have a deficiency in protein 4.1 levels in the RBC membrane.

RBCs rapidly react to oxidative stress through very intense Tyr phosphorylation of band 3. It was found that the phosphorylation of band 3 affects its interactions with the cytoskeleton inducing membrane destabilization [Ferru E., et al., 2011]. It was suggested that band 3 acts as a redox sensor regulating its phosphorylation and that hemichromes leading to the protracted phosphorylation of band 3 may trigger a cascade of events finally leading to hemolysis [Pantaleo A., et al., 2016]. The sex- and age-related alterations in RBCs membranes proteins (especially in women of menopausal age) were Identified [Pruidze N, et al., 2015].

The majority of RBC membrane protein ratios, including band 3/spectrin, were more elevated in critically ill patients (nonseptic and septic) than in healthy volunteers [Serroukh Y., et al., 2012].

Alternations of RBCs membrane proteins during diabetes

It has been shown that the topographical nanostructure of the RBC membrane and their roughness can be classified as independent morphological parameters of the membrane describing its both primary and altered structure and functional status [Buys, 2013; Bhise SS, et al., 2020]. A difference in diameter, height, and concave depth of RBCs between the healthy and diabetic individuals was detected. Measurement of surface roughness indicated the alterations related to the cytoskeletal matrix and the connections between band 3 and 4 proteins with the matrix [Buys, 2013].

In diabetes, RBC membranes are affected by chronic exposure to glucose, and several biochemical modifications are triggered, with subsequent structural and functional disruption of the cell, which is further involved in the pathogenesis of diabetes and its complications.

In patients with diabetes type-1, decreased content of negatively charged glycophorin C and high molecular fraction proteins (weight of 55- 260 kDa) in RBCs membrane was detected [Gabunia T, 2015]. Decrease of glycophorin content may not be crucial to the RBC mechanical stability, deformability, and shape change [de Oliveira Sofia, 2010] but may induce adhesion of RBCs to the endothelium and disorders of blood circulation. Low content of heavy proteins (spectrin, alpha (260 kDa) and beta (225 kDa), ankyrin (215 kDa), Band 4.1 (78 kDa), band 4.2 (72 kDa), band 3 (90 - 100 kDa) proteins, associated to the cytoskeleton, which play a very important role in the regulation of the flexibility and rigidity of RBC, involved in the regulation of RBCs mechanical stability, deformability, and shape, may induce disorders of their deformability [Gabunia T, 2015].

Structural alterations of the RBCs membrane proteins (increased mobility of band 3, an aberrant high molecular weight (> 255 kDa), and a low molecular weight (42 kDa) proteins, increase of the 3-band protein, and reduction of spectrin content) were shown in patients with diabetic retinopathy. With the increased duration and severity of diabetes, the degree of impairment in the protein fraction of RBCs membranes increased [Petropoulos IK., et al., 2007]. Increase the content of RBC several glycated membrane proteins (actin, protein 4.1, spectrin alpha, spectrin beta chain, protein band 4.2, and ankyrin) was found in individuals with abnormal glucose tolerance and diabetes compared to control, but it was not found significant differences in RBC between patients with diabetes and impaired glucose tolerance. It has been found heavy glycosylation of the cytoskeletal proteins and oxidative damage of spectrin in RBCs from diabetic patients. The main non-enzymatic protein glycation products might be useful sensitive biomarkers for long-term glycemic control, early diagnosis, or disease progression.

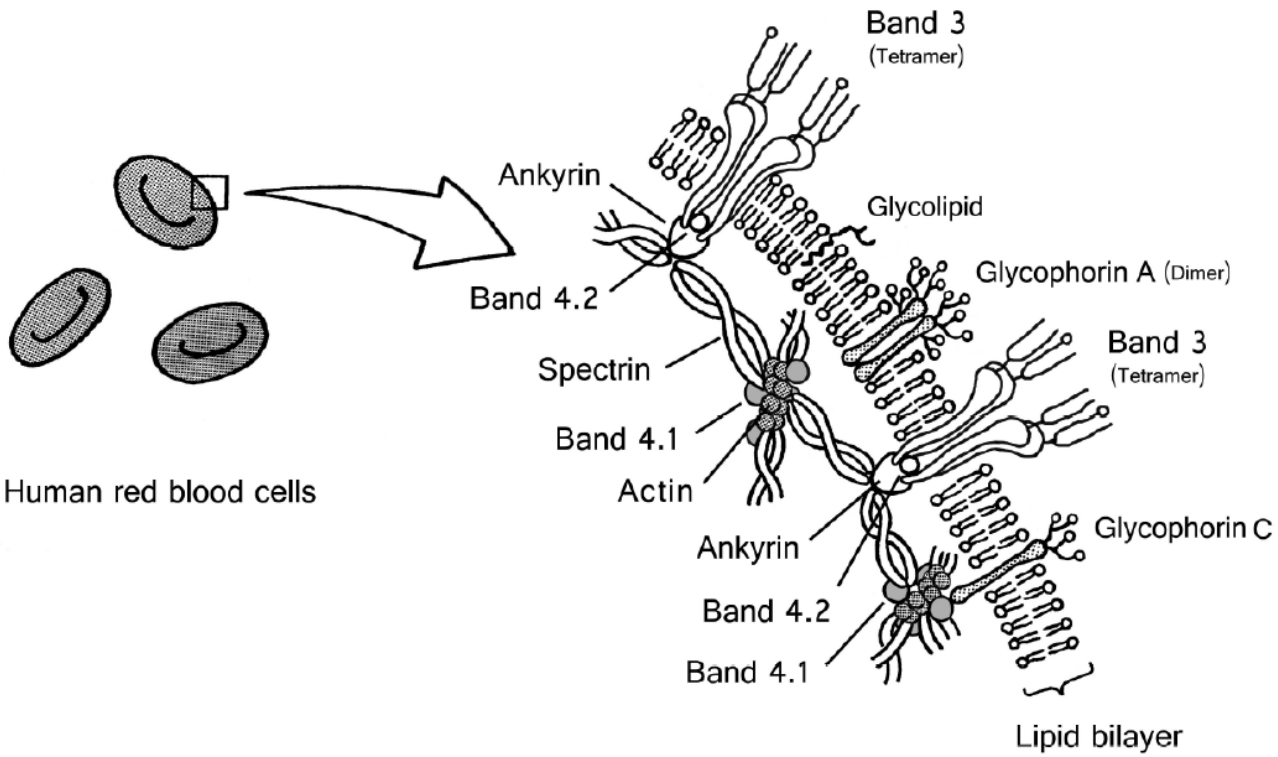
Late glycation products (advanced glycated end products (AGEs) were linked with diabetic complications and are important for clinicians in monitoring the progression of the disease. An increase of 3-fold of AGEs was identified in the RBC peripheral membrane proteins in the diabetic patient's group compared with healthy controls, and these results correlated with the glycated hemoglobin levels. It was suggested the implication of AGEs in the decreased deformability and reduced membrane fluidity in the diabetic RBC. It has been observed that in diabetic patients' RBCs with high levels of AGEs on the surface are capable to interact with an immunoglobulin receptor for AGE (RAGE)) expressed on endothelial cells, thus triggering a vascular dysfunction involved in the diabetic vascular complications [Gabreanu G.R., Angelescu S.. 2016; Singh VP., et al., 2014].

Several advanced glycation end products, such as imidazoline, crossline, pyrrolidine, N- epsilon-(Carboxymethyl) lysine, and pentosidine, linked with the development of diabetic complications were identified in the investigation of blood samples from diabetic patients. For example, serum pentosidine was found elevated in both diabetic nephropathy and retinopathy, and it was suggested as a useful biomarker for microvascular complications in type 2 diabetes. Crossline (fluorescent advanced glycated end product) level in RBC membrane proteins was increased by 1.6-fold in diabetic patients compared with healthy controls; its significantly high level was detected in the patients with complicated diabetes (non-proliferative retinopathy, proliferative retinopathy, diabetic nephropathy). Further research on the erythrocyte membrane could provide new biomarkers for monitoring diabetes and its complication [Gabreanu G.R., Angelescu S.. 2016].

Conclusion

Tightly association of several RBCs' membrane proteins disorders with different pathological processes was identified. In diabetes, RBC membranes are affected by chronic exposure to glucose, triggering their biochemical modifications (glycosylation, oxidation), with subsequent structural and functional disruption of the cell (disorders of their deformability, adhesion to the endothelium), which is further involved in the pathogenesis of diabetes and its complications. The alterations of RBCs membrane proteins might be useful sensitive biomarkers for long-term glycemic control, early diagnosis, or monitoring of disease progression.

Figure 1. Schematic structure of human RBCs membrane (Takahiko Aoki, 2017)



References

1. Pasini EM, Kirkegaard M, Mortensen P, Lutz HU, Thomas AW, Mann M. In-depth analysis of the membrane and cytosolic proteome of red blood cells. Blood. 2006 Aug 1;108(3):791-801.
2. Steinem C. Cell Membrane. The Red Blood Cell as a Model. Von Yoshihito Yawata. Angewandte Chemie. 2003 Nov;115(45):5699-700.
3. De Oliveira S, Saldanha C. An overview about erythrocyte membrane. Clinical hemorheology and microcirculation. 2010 Jan 1;44(1):63-74
4. Deuticke B. Membrane lipids and proteins as a basis of red cell shape and its alterations. InRed cell membrane transport in health and disease 2003 (pp. 27-60). Springer, Berlin, Heidelberg.
5. Piagnerelli M, Boudjeltia KZ, Vanhaeverbeek M, Vincent JL. Red blood cell rheology in sepsis. Applied Physiology in Intensive Care Medicine. 2009:273-82.
6. Winzler,, R. J. 1969. A glycoprotein in human erythrocyte membranes. In Red Cell Membrane Structure and Function. G. A. Jamieson and T. J. Greenwalt, editors. J. B. Lippincott Co., Philadelphia. 157.
7. Steck tl. the organization human red blood a review of proteins in cell membrane the theodore l. steck the journal of cell biology • volume 62, 1974, 3-19.
8. Patra M, Mukhopadhyay C, Chakrabarti A. Probing conformational stability and dynamics of erythroid and nonerythroid spectrin: Effects of urea and guanidine hydrochloride. PloS one. 2015 Jan 24;10(1):e0116991.
9. Aoki T. A comprehensive review of our current understanding of red blood cell (RBC) glycoproteins. Membranes. 2017 Dec;7(4):56.
10. Pantaleo A, Ferru E, Carta F, Valente E, Pippia P, Turrini F. Effect of heterozygous beta thalassemia on the phosphorylative response to Plasmodium falciparum infection. Journal of proteomics. 2012 Dec 5;76:251-8.
11. Telen MJ. Erythrocyte adhesion receptors: blood group antigens and related molecules. Transfusion medicine reviews. 2005 Jan 1;19(1):32-44.
12. An X, Salomao M, Guo X, Gratzer W, Mohandas N. Tropomyosin modulates erythrocyte membrane stability. Blood. 2007 Feb 1;109(3):1284-8.
13. Ferru E, Pantaleo A, Carta F, Mannu F, Khadjavi A, Gallo V, Ronzoni L, Graziadei G, Cappellini MD, Turrini F. Thalassemic erythrocytes release microparticles loaded with hemichromes by redox activation of p72Syk kinase. Haematologica. 2014 Mar;99(3):570.
14. Ferru E, Giger K, Pantaleo A, Campanella E, Grey J, Ritchie K, Vono R, Turrini F, Low PS. Regulation of membrane-cytoskeletal interactions by tyrosine phosphorylation of erythrocyte band 3. Blood, The Journal of the American Society of Hematology. 2011 Jun 2;117(22):5998-6006.
15. Pantaleo A, Ferru E, Pau MC, Khadjavi A, Mandili G, Mattè A, Spano A, De Franceschi L, Pippia P, Turrini F. Band 3 erythrocyte membrane protein acts as redox stress sensor leading to its phosphorylation by p72 Syk. Oxidative medicine and cellular longevity. 2016 Jan 1;2016.
16. Pruidze N, Khetsuriani R, Sujashvili R, Ioramashvili I, Arabuli M, Sanikidze T. ALTERATIONS OF PROPERTIES OF RED BLOOD CELLS MEMBRANES PROTEINS OF DIFFERENT AGE AND SEX VOLUNTEERS. Georgian Medical News. 2015 Jul 1(244-245):110-5.
17. Serroukh Y, Djebara S, Lelubre C, Zouaoui Boudjeltia K, Biston P, Piagnerelli M. Alterations of the erythrocyte membrane during sepsis. Critical care research and practice. 2012 Oct;2012.
18. Buys AV, Van Rooy MJ, Soma P, Van Papendorp D, Lipinski B, Pretorius E. Changes in red blood cell membrane structure in type 2 diabetes: a scanning electron and atomic force microscopy study. Cardiovascular diabetology. 2013 Dec;12(1):1-7.
19. Bhise SS, Rao JR, Hegde MV, Katyare SS. Compositional alterations in erythrocyte membranes in Type II diabetes. Indian Journal of Experimental Biology (IJEB). 2020 Sep 30;58(10):671-9.
20. Gabunia T, Turabelidze-Robaqidze S, Sujashvili R, Ioramashvili I, Gogebashvili N, Sanikidze T. ALTERATIONS OF RBC MEMBRANE PROTEINS IN DIABETIC PATIENTS WITH AND WITHOUT PERIODONTITIS. Georgian Medical News. 2015 Nov 1(248):39-45.
21. Petropoulos IK., Panagiotis Margetis, Marianna H. Antonelou, John X. Koliopoulos, Sotirios P. Gartaganis, Lukas H. Margaritis, Issidora S. Papassider. Structural alterations of the erythrocyte membrane proteins in diabetic retinopathy Archive for Clinical and Experimental phthalmology 245.8 (Aug 2007): 1179 -88.
22. Gabreanu GR, Angelescu S. Erythrocyte membrane in type 2 diabetes mellitus. Discoveries. 2016 Apr;4(2).
23. Singh VP, Bali A, Singh N, Jaggi AS. Advanced glycation end products and diabetic complications. The Korean journal of physiology & pharmacology. 2014 Feb 1;18(1):1-4.