An update on red blood cell storage lesions, as gleaned through biochemistry and omics technologies

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Red blood cell (RBC) aging in the blood bank is characterized by the accumulation of a significant number of biochemical and morphologic alterations. Recent mass spectrometry and electron microscopy studies have provided novel insights into the molecular changes underpinning the accumulation of storage lesions to RBCs in the blood bank. Biochemical lesions include altered cation homeostasis, reprogrammed energy, and redox metabolism, which result in the impairment of enzymatic activity and progressive depletion of high-energy phosphate compounds. These factors contribute to the progressive accumulation of oxidative stress, which in turn promotes oxidative lesions to proteins (carbonylation, fragmentation, hemoglobin glycation) and lipids (peroxidation). Biochemical lesions negatively affect RBC morphology, which is marked by progressive membrane blebbing and vesiculation. These storage lesions contribute to the altered physiology of long-stored RBCs and promote the rapid clearance of up to one-fourth of long-stored RBCs from the recipient's bloodstream after 24 hours from administration. While prospective clinical evidence is accumulating, from the present review it emerges that biochemical, morphologic, and omics profiles of stored RBCs have observable changes after approximately 14 days of storage. Future studies will assess whether these in vitro observations might have clinically meaningful effects.

In most countries, the shelf life of red blood cells (RBCs) is limited to 42 days. However, results from retrospective clinical trials have hinted at a correlation between untoward consequences in certain categories of recipients (e.g., traumatized, critically ill, or perioperative patients) and transfusion of RBCs stored longer than 14 days.[1,2] While clinical prospective evidence is still missing or inconclusive,[3] an accumulating body of evidence indicates that biochemical and morphologic lesions to stored RBCs tend to accumulate soon after the

ABBREVIATIONS: AE1 = Anion Exchanger 1; GSH = glutathione; miRNA(s) = micro RNAs; PPP = pentose phosphate pathway; PS = phosphatidylserine; ROS = reactive oxygen species.

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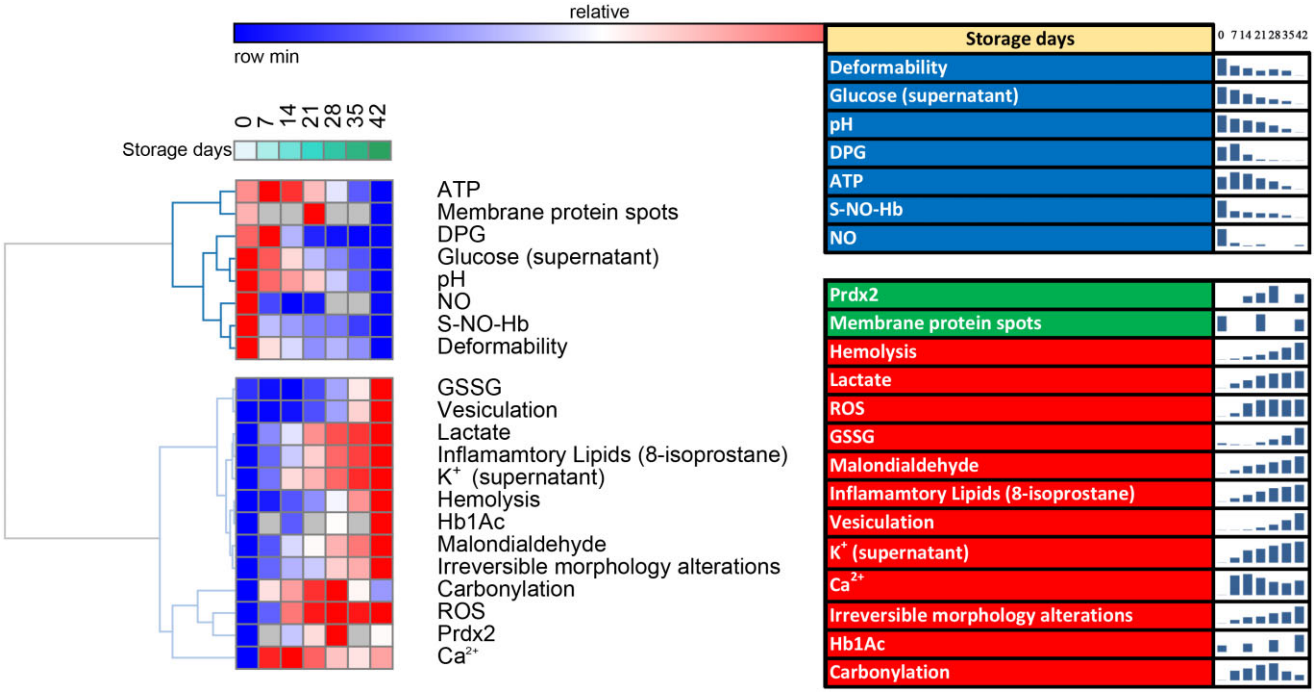

Fig. 1. Dynamics of the main biochemical lesions during RBC storage. Time course Z-score normalized quantitative changes of biochemical and morphologic variables during storage durations. Heat maps (left) and sparklines (right) have been graphed based on reelaboration of originally published results.[8,9,19,21,26,59,64,65] Quantitative changes are graphed either in blue (decrease) or in red (increase) against normalized values.

second week of storage (Fig. 1).[4-9] The bulk of this evidence comes from the application of mass spectrometry–based metabolomics, proteomics, and lipidomics to the field of transfusion medicine, as we will discuss in this review.

Omics disciplines are characterized by the systematic determination and quantification of broad classes of molecules, such as metabolites (low-molecular-weight compounds below 1.5 kDa), proteins,[8] and lipids.[10] Data from multiple omics platforms can be then integrated through bioinformatic approaches and mathematical modeling to obtain a systems biology level of understanding.[11]

AGING IN VIVO AND IN VITRO

A deeper understanding of the molecular mechanisms driving RBC aging in the blood bank should aid in the design of new storage strategies to extend the shelf-life of RBCs. However, these mechanisms have been only partially disclosed and are incompletely understood.[4-7]

RBC aging in vivo and in vitro are characterized by distinct mechanisms.[7,12,13] In vivo circulating RBCs have an approximate life span of 120 days.[13] Approximately 1×10^{11} RBCs are generated every day and cleared from the bloodstream by residential macrophages in the reticuloendothelial system, through a synchronized mechanism underpinning their generation and senescence in peripheral blood.[7,13,14]

Circulating RBCs are characterized by heterogeneous RBC populations,[15,16] and RBC populations are differentially affected by injuries in vivo[13,16] or storage in the blood bank.[17] Although up to 25% of the transfused RBCs is rapidly removed from the bloodstream of the recipient, RBCs that survive the first 24 hours in circulation have a normal or near-normal survival.[18] This evidence strengthens the case for an increased susceptibility of certain RBC populations to the so-called "storage lesions."[17,18]

While in vivo aging of RBCs culminates with senescence, aging in vitro has been also associated with eryptosis, a controversial process that closely mimics the programmed cell death of nucleated cells (apoptosis).[14] Eryptotic phenomena in vivo result from injury or (oxidative) stress to RBCs.[16] Under blood bank conditions, ensuing of eryptosis is tied to storage lesions (Fig. 2).[13]

In the following paragraphs we will relate the accumulation of biochemical and morphologic storage lesions to the impaired physiology and functionality of RBCs. Focus will be on biochemical and omics studies on oxygen transport, cation homeostasis, energy and redox metabolism. The association of these storage-influenced biochemical variables to the compromised protein and structural integrity of long-stored RBCs will be explored. Finally, we will discuss the donor variability issues, as they

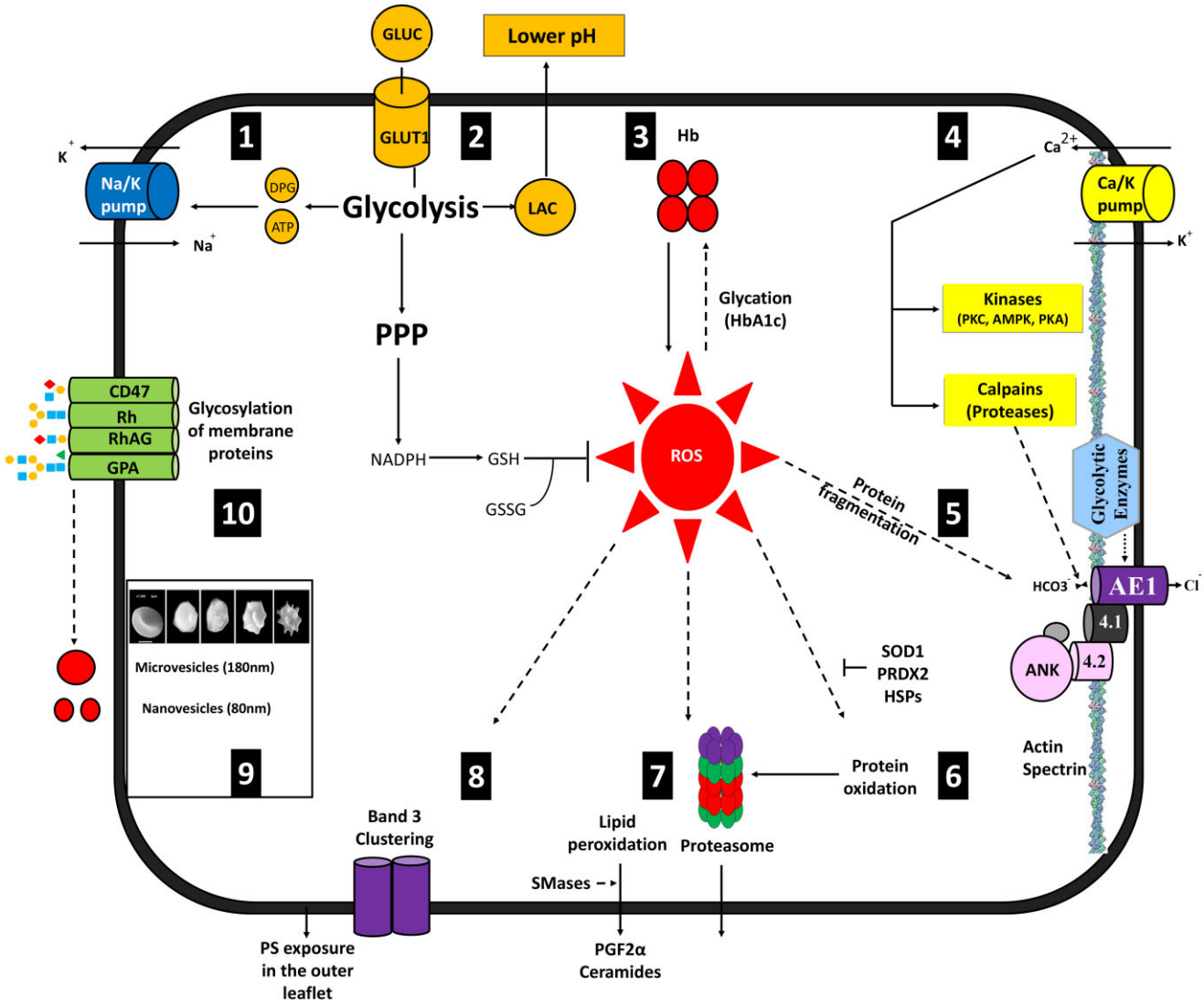

Fig. 2. An overview of the main biochemical changes of in vitro aging RBCs under blood bank conditions. In clockwise order: (1) Cation homeostasis is influenced by low temperatures and depletion of ATP and DPG. (2) Glucose is consumed through glycolysis, as to produce ATP and lactate (LAC) and promote pH lowering. (3) Low temperatures and oxidative stress (Hb-mediated Fenton reactions) promote the PPP and impair GSH homeostasis. (4) Alterations to calcium homeostasis (as well as of cAMP and AMP) promote kinases (e.g., PKC, PKA, AMPK) or proteolytic enzymes (such as calpains) targeting Band 3 (AE1) and structural proteins. (5) AE1 modulates pH through the chloride shift and indirectly influences Hb-oxygen affinity and gas exchanges. Fragmentation of the cytosolic domain of AE1 (by ROS, calpain, and caspases) displaces glycolytic enzymes and structural proteins (ankyrin [ANK], Band 4.2, and Band 4.1). (6) Protein oxidation is partly challenged by antioxidant defenses (SOD1, PRDX2) and chaperone molecules (heat shock proteins [HSPs]). Still, storage promotes redox modifications to proteins (carbonylation, glycation of Hb, fragmentation) and lipids. (7) Storage affects degradation of proteins (via the proteasome, extruded in the supernatant) and lipids (sphingomyelinase-dependent accumulation of ceramides). (8) Storage promotes membrane accumulation of AE1 clusters, exposure of PS in the outer leaflet, and lipid raft formation that could alter the RBC proimmunogenic potential. (9) These alterations affect membrane deformability, increase osmotic fragility, and promote vesiculation events, a process through which micro- and nanovesicles are shed as to eliminate irreversibly altered proteins (among which traces of glycolytic enzymes). (10) Exocytic vesicles are enriched with Hb, lipid raft proteins, and membrane portions (also exposing common antigens).

influence transfusion outcomes and hitherto hampered omics investigators from drawing universally valid conclusions.

OXYGEN TRANSPORT

Administration of transfusion therapies in the intensive care setting is associated with the need to restore tissue oxygenation, volemia, and blood viscosity in response to hemorrhagic shock. Long-stored human RBCs are characterized by a higher oxygen affinity, since pO_2 is essentially unchanged between 3 hours and 14 days, whereas hemoglobin (Hb) O_2 saturation increases steadily throughout storage duration up to 99% by Storage Day 42.[19] Consistently, a storage-dependent increase in O_2 affinity was recently confirmed,[20] although in vitro interactions with oxygen were largely preserved through 42 days of storage.[20] Such effects are promoted by the storage-dependent consumption of high-energy phosphate compounds (adenosine triphosphate [ATP] and 2,3-diphosphoglycerate [2,3-DPG]).[21] These compounds are known to act as allosteric effectors as they stabilize the "T" (deoxygenated) state of Hb and thus their decrease positively affects Hb-oxygen affinity.[20] However, it is worth noting that oxygen offloading from Hb is promoted by intracellular acidification (Bohr effect), a condition that is observed during storage in the blood bank,[22] as a result of ongoing glycolysis (Embden-Meyerhoff energy metabolism pathway).[21] Conversely, the decrease in pH has a negative feedback on glycolysis.[23] Storage is also accompanied by deregulated S-nitrosylation of Hb at β93cys, suggesting a likely compromised "hypoxic vasodilation" capacity of longer-stored RBCs.[19]

However, it has recently been concluded that, although fresh RBCs might be superior to long-stored RBCs, increased oxygen affinity of "older" RBCs may provide a benefit in hemorrhagic shock resuscitation.[24]

CATION TRANSPORT

Hypothermia during storage in the blood bank is known to negatively influence the activity of cation transporters.[25] Older RBCs display altered Na^+/K^+ fluxes,[9,19] resulting in the supernatant accumulation of potassium, a pitfall compromising transfusions to certain recipients, such as pediatric patients. Impaired potassium homeostasis is also linked to the progressive increase of intracellular ionic calcium.[21,26,27] Depletion of ATP promotes calcium build-up in the cytosol,[21] since internal Ca^{2+} is subjected to metabolic control via an ATP-dependent extrusion mechanism (Ca^{2+} pump). As a consequence, intracellular calcium accumulation triggers the opening of the Ca^{2+}-dependent K^+ channel, other than the activation of calcium-dependent proteases (such as μ-calpain) while promoting the onset of apoptosis-like phenomena.[3] However, eryptosis in long-stored RBCs is mainly tied to starvation (depletion of high-energy phosphate compounds) rather than to calcium alone.[28] Calcium loading also results in dose-dependent decreases in reduced glutathione (GSH) levels in rabbit RBCs[29] and promotes glutathione *S*-transferase migration to the cell membrane in human RBCs.[30]

ENERGY AND REDOX METABOLISM

Efficiency of energy metabolism is measured by the rate of high-energy phosphate compound generation. These metabolites serve as energy tokens to be spent on the preservation of cellular homeostasis. For example, ATP levels influence membrane stability and thus RBC survival.[31] However, alterations to DPG, ATP, and cation imbalances are rapidly reversible upon transfusion of RBCs in the bloodstream of the recipients.[32]

Energy and redox metabolism are intimately connected in RBCs, which can rely on glycolysis to generate approximately 90% of cell energy through anaerobic oxidation of glucose.[7] In response to high oxygen saturation and oxidative stress, glucose catabolites are channeled through the pentose phosphate pathway (PPP) to fuel the generation of NADPH and maintain GSH redox poise. Branching from glycolysis, the Rapoport-Luebering shunt interconverts the 1,3- and 2,3-isoforms of DPG, thereby connecting energy metabolism to Hb-oxygen affinity.[7] Glycolysis also influences the $NADH/NAD^+$ ratio. NADH contributes to redox homeostasis by promoting ferric heme iron reduction back to the ferrous state, a reaction catalyzed by the NADH-dependent enzyme cytochrome b5 reductase in the methemoglobin reduction pathway.[7] As the list of proteins in the RBC proteome rapidly expands through modern proteomics approaches (recently extended to 2289 entries and counting),[33,34] novel or hitherto underinvestigated RBC metabolic pathways might emerge in the future as key players in the accumulation of storage-triggered metabolic lesions. In analogy to cancer-induced alterations to cell metabolism, examples might be represented by serine and glutamine metabolism and their indirect role in GSH homeostasis.[35]

Energy metabolism

RBC storage is characterized by the progressive depletion of ATP and DPG reservoirs, a phenomenon facilitated by the negative influence of hypothermia on enzyme activities. Nevertheless, storage is accompanied by the constant accumulation of lactate in the supernatants.[21] Storage of RBCs in CPD-SAGM,[21,36,37] MAP,[38] AS-1,[39] or PAGGGM[40,41] results in the early accumulation of glycolytic intermediates during the first 2 weeks of storage and rapid decrease soon afterward. These observations likely arise from a metabolic modulation that promotes a shift toward the

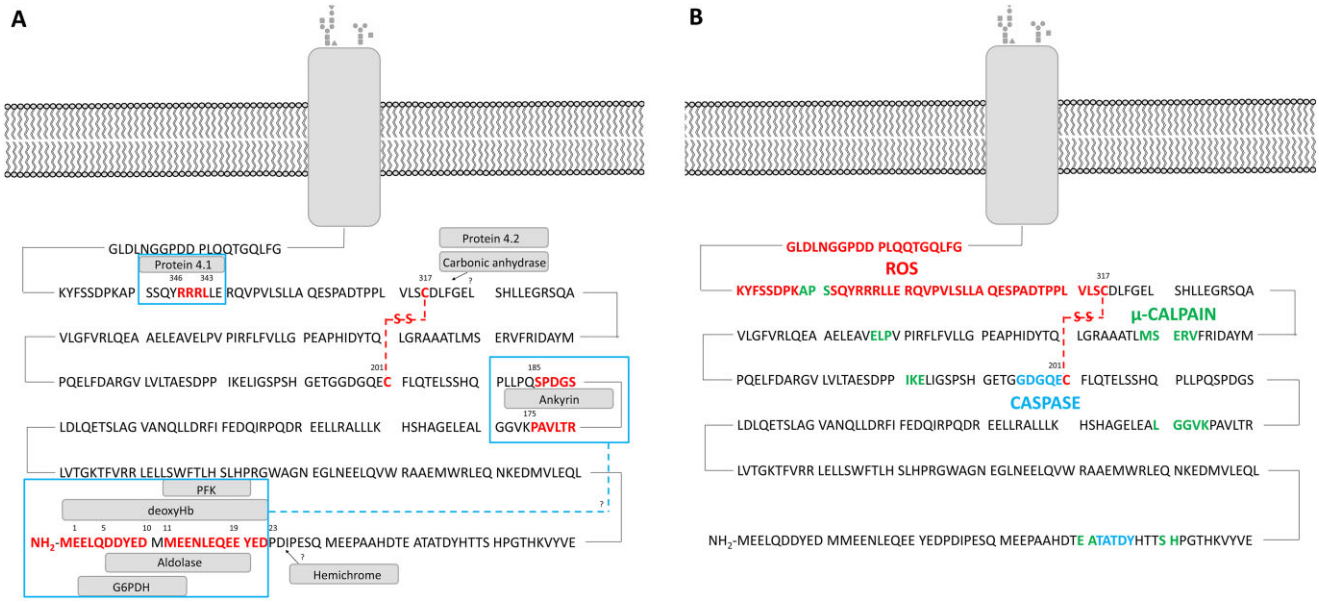

Fig. 3. Band 3 and the transport metabolon. In A, glycolytic and structural enzymes on the CDB3 and the relative binding sites (red bold font). In B, amino acid sequence targeted by ROS (red bold font), caspase (blue bold font), and μ-calpain (green bold font) during storage in the blood bank (literature-based or in silico prediction via GPS-CCD—http://ccd.biocuckoo.org/down.php).

PPP via partial glycolytic blockade. However, the ratio of metabolic intermediates of the PPP and glycolysis[21,36] indicates that such a compensatory mechanism, whether confirmed, might only be transient and progressively impaired[35] from the second storage week onward.[21] Alkaline additives or rejuvenation solutions are currently under evaluation as they have been reported to better preserve, or to replenish, ATP and DPG reservoirs even upon extended storage.[42]

Redox metabolism

Storage of RBCs results in the progressive deregulation of the redox poise, as it is accompanied by decreased GSH and increased GSSG levels.[21] GSH homeostasis is negatively affected by a decline in GSH anabolism, resulting from a reduced uptake[43] and increased efflux[44] of amino acid precursors (glutamate, glutamate-precursor glutamine, glycine, and cysteine), secondary to a storage-dependent decrease in ATP concentrations.[43]

Reactive oxygen species (ROS) in the form of hydroxyl radicals and superoxide are generated through Haber Weiss and Fenton reactions from heme iron.[45] ROS tend to reach a maximum within the first 2 weeks of storage, both in leukofiltered and in nonleukofiltered units (though to a lesser extent in the former).[8,26,46,47]

THE PROGRESSIVE LOSS OF METABOLIC MODULATION IS ATTRIBUTABLE TO LESIONS TARGETING BAND 3

The Anion Exchanger 1 (AE1; Band 3) is the most abundant RBC membrane protein (approx. 1×10^6 copies/cell). AE1 lies at the crossroads between anion homeostasis, gas transport, and metabolic modulation.[7] The main role of this protein is to promote the so-called "chloride shift," a process resulting in the exchange of cellular HCO_3^- with plasma Cl^-. In so doing, AE1 participates with carbonic anhydrase to modulate gas transport (O_2 release and CO_2 uptake). By favoring the conversion of the weak acid H_2CO_3 to the strong acid HCl, AE1 contributes to the acidification of the intracellular pH. The transient acidification triggered by AE1 activity boosts O_2 release from Hb (Bohr effect) and oxygen supply to those tissues producing more CO_2 (lactate-rich acidic districts). However, AE1 is not only tied to gas transport homeostasis, since its N-terminal cytosolic domain provides a docking site for a series of structural proteins and glycolytic enzymes (e.g., phosphofructokinase, aldolase, glyceraldehyde 3-phosphate and lactate dehydrogenases; Fig. 3A).[48,49] These interactions result in the assembly of a multiprotein complex often referred to as the "respiratory metabolon." Biochemical studies have highlighted a role for the negatively charged residues at the N-terminal cytosolic domain in mediating enzyme-AE1 interactions. These interactions are further promoted by phosphorylation of tyrosine 8 and 21.[50] Negative charges in this region also serve to stabilize deoxyhemoglobin,[49] whose binding to AE1 triggers the release and thus reactivation of otherwise bound-inhibited glycolytic enzymes (Fig. 4), providing an oxygen-dependent metabolic modulation.[49]

AE1 is also involved in redox homeostasis, as it also interacts with peroxiredoxin 2,[51] a scavenger of

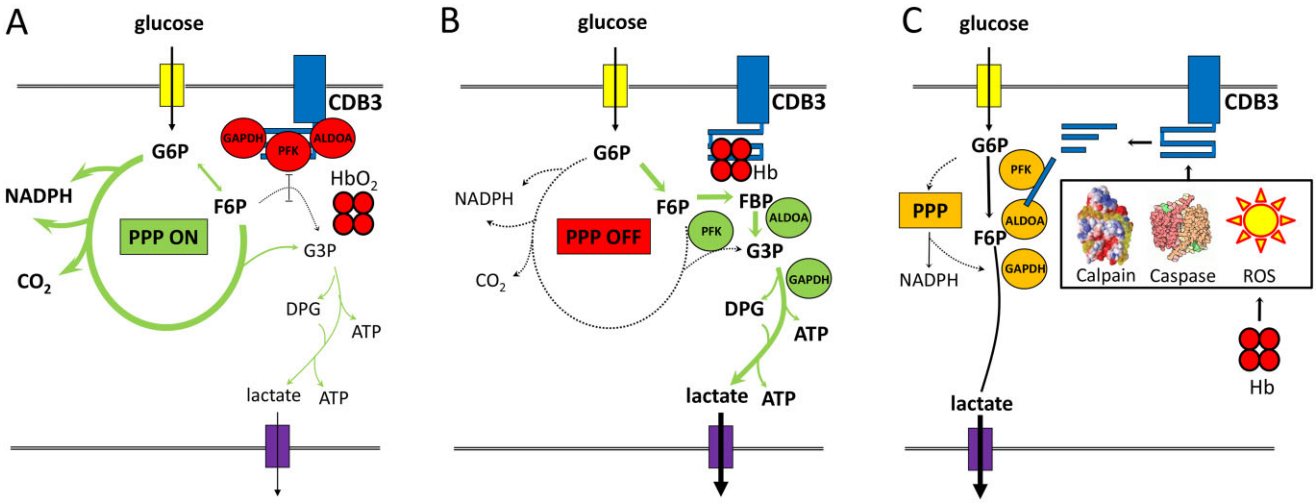

Fig. 4. Oxygen-dependent metabolic modulation by Band 3 and storage lesions. Oxygen-dependent metabolic modulation is mediated by the competitive binding of deoxyhemoglobin and glycolytic enzymes phosphofructokinase (PFK), aldolase (ALDOA), and glyceralhdehyde-3-phosphate dehydrogenase (GAPDH) to the cytosolic domain of Band 3 (CDB3). In A, enzymes are bound and inhibited (high oxygen saturation and/or oxidative stress). This mechanism promotes a metabolic shift from glycolysis to the PPP. In B, deoxyhemoglobin binding to the CDB3 releases glycolytic enzymes and promotes glycolysis (low oxygen saturation). In C, fragmentation of the CDB3 is triggered by the activation of caspases, calpain, and ROS. Fragmentation of CDB3 results in the impairment of the oxygen-dependent metabolic modulation of RBCs. Enzymes are shown in red (A—inhibited), green (B—active), or orange (C—enzyme activity potentially influenced by storage lesions).

low-level hydrogen peroxide.[52] Progressive translocation of peroxiredoxin 2 to the RBC membrane during storage in the blood bank has been documented,[52] both in leuko- or in nonleukoreduced units,[8,26,53] and proposed to be a biomarker of autologous blood transfusions as illicit doping practices for endurance sport athletes.[17]

Clustering of the extracellular regions of AE1 might contribute to the removal of transfused RBCs from the bloodstream of the recipient, by stimulating binding of Band 3 antibodies and removal by the spleen and liver macrophages.[54] Additionally, alterations to the oligomeric state of AE1 have been reported to precede membrane phospholipid loss during storage of RBCs in the blood bank.[55] Oligomerization of AE1 might be promoted by oxidative stress, since oxidized and poorly glycosylated AE1 is selectively phosphorylated by Syk kinase, which in turn promotes the formation of large membrane clusters in normal and glucose-6-phosphate dehydrogenase–deficient RBCs.[50]

Finally, AE1 is targeted by intracellular proteases (such as caspases) and ROS, which results in the generation of distinct fragments of AE1 (24 and 35 kDa, respectively; Fig. 3B).[56] Of note, caspase-3 activation is consistent with the storage-dependent triggering of a Fas/caspase-driven death program.[57]

OXIDATIVE DAMAGE TO PROTEINS: FRAGMENTATIONS, CARBONYLATIONS, AND NONENZYMATIC GLYCATION

Storage-dependent oxidation of proteins results in at least three main documented events: 1) increased protein fragmentation, membrane migration, or externalization;[8,46,47,58-62] 2) increased protein carbonylation;[8,61-64] and 3) increased (non-)enzymatic glycosylation of cytosolic[65] and membrane proteins.[66] Alterations to RBC cytosolic and membrane proteins during storage have been extensively documented through proteomics technologies,[8,46,58-62] and include: 1) the fragmentation of structural proteins (spectrin, ankyrin, AE1, and Band 4.1 and 4.2—triggered by either proteases or ROS); 2) membrane accumulation of Hb, antioxidant enzymes (peroxiredoxin 2), and chaperones; and 3) cytosolic decrease of transglutaminase-2, β-actin, and copper chaperone for superoxide dismutase.[8,46,57-62] Remodeling of the cytoskeleton has been appreciated through the observed relocation of vesicle-associated membrane fusion proteins (SNAPs)[8] and the decrease in RBC membrane content of lipid raft-associated proteins flotillins and stomatin.[60] Stored RBCs also tend to exocytose the otherwise functional proteasome,[67] which is likely indicative of an impaired capacity of the

ubiquitination system in older RBCs,[68] thus limiting the removal of irreversibly damaged proteins.

Aging of RBCs, both in vivo and in vitro, also promotes a conformational change to CD47, a "do not eat me" signal for RBC phagocytosis as it interacts with the inhibitory immunoreceptor SIRPα expressed by macrophages.[69] Senescent and long-stored RBCs display CD47 that has undergone a conformational change that triggers its binding to thrombospondin-1. This promotes RBC phagocytosis by human red pulp macrophages and is thus associated with a shortened survival of transfused older RBCs.[69]

Carbonylation of RBC proteins, a hallmark of oxidative lesions, increases until the fourth week of storage[8,61,64] (especially in nonleukoreduced units)[26] and then decreases by the end of the storage period. The decrease is due either to proteasome activity or vesiculation,[63,70] two likely self-protective and/or age-dependent mechanisms that will be further discussed below.

ROS-mediated nonenzymatic glycosylation of proteins (i.e., glycation) has been reported to target the most abundant cytosolic (i.e., Hb α and β-chains)[65] and membrane proteins.[66] Such phenomena might be exacerbated by the excessive glucose found in anticoagulation and additive solutions (ASs). Indeed, by the end of the storage period, glucose levels in the supernatants of CPD-SAGM–stored RBCs are approximately 12 ± 1 mmol/L,[41] which is still higher than circulating glucose in diabetic patients (subjects with a consistent glycemia above 7 mmol/L are generally deemed to be diabetic).[71] Increased rates of enzymatic glycosylation of RBC membrane proteins is a potentially adverse event,[66] as membrane-associated carbohydrate structures contribute to alter rheologic properties and the proimmunogenic potential of transfused RBCs.

OXIDATIVE STRESS TO LIPIDS

Aging of RBCs results in the progressive accumulation of oxidative stress markers in the lipid fraction (in the form of malondialdehyde[8,72] or prostaglandins, such as 8-isoprostane).[21] Again, a factor contributing to lipid peroxidation might be represented by the elevated glucose loading in collection and storage solutions, which fuels glucose autoxidation.[73] Accumulation of high levels of prostaglandins or oxidized lipids in the supernatants of long-stored RBCs are likely to promote adverse events (e.g., transfusion-related acute lung injury) or inflammatory responses in the recipients.[74,75]

Like the proteome, the RBC lipidome is diet (and thus donor) dependent and subject to stability, in that mature RBCs are devoid of de novo long-chain fatty acid synthesis enzymes.[76] In this view, alterations to the lipidome are regarded to be irreversible. One of the earliest observed alterations to the RBC lipidome during storage in the blood bank is the progressive increase in membrane phospholipid asymmetry, owing to consumption of ATP reservoirs, which results in the apoptosis-like[14] externalization of phosphatidylserine (PS) to the outer leaflet of the plasma membrane.[77]

Recent lipidomics studies[78,79] indicated that long-stored RBCs display higher levels of ceramides, which are released from cell membrane sphingomyelins by an acid sphingomyelinase.[80] The stimulation of sphingomyelinase is dependent on the platelet (PLT)-activating factor, which is in turn generated from cell membrane lipids by an osmotic shrinkage-dependent phospholipase.[14]

MORPHOLOGIC CHANGES AND MEMBRANE EXOVESICULATION

Physiologic shape, protein interactions, and surface area-to-volume ratio jointly determine the biomechanical properties of circulating RBCs that are critical for their survival.[81] Unlike the in vivo aged RBCs, that progressively become smaller and more dense,[13] storage in the blood bank is associated with an early and probably reversible increase in RBC volume.[26,82] This phenomenon theoretically affects cell deformability.[82] Visible shape abnormalities appear soon after the first week of storage and occur in about one-third of the original RBC population by the end of the storage period.[9,26,83] These changes result in loss of the smooth biconcave disc morphology and acquisition of altered morphologies, either reversible or irreversible (echinocytes and stomatocytes or spherocytes).[9,83]

Reversibility of morphologic alterations is inversely proportional to storage duration and is dependent on membrane–cytoskeletal interactions.[84] These in turn are modulated by cellular metabolism, ATP levels, and cation homeostasis. Morphologic variation seems to be closely associated with storage-related disturbances in cellular deformability,[85] osmotic fragility,[9] mechanical fragility,[86] and rheologic properties.[83,87] To some extent, morphologic lesions can be prevented by leukoreduction[88] or reversed by rejuvenation.[86]

Irreversible morphologic alterations are those involving loss of significant portions of the RBC membrane through exovesiculation. Microvesicles are released from the tips of the echinocytic spines of RBCs transformed beyond the early spheroechinocyte stage.[89] Microvesicles are a measure of RBC damage during storage as well as a potential source of mediators that lead to adverse posttransfusion effects. In extreme spherocytosis, the loss of all extra surface implies critically compromised cell surface-to-volume ratio and deformability, which aggravate both in-bag hemolysis and posttransfusion recovery.[90]

RBC-shed microparticles have also been considered as biomarkers of storage quality. Indeed, they are important carriers of extracellular Hb[60,62,91,92] and can contribute

to immunogenic, proinflammatory, procoagulant, thrombogenic, and NO scavenging activities.[93-98] Vesicles shed from apoptotic RBCs are characterized by PS externalization.[99]

The rate of RBC vesiculation increases after the second week of storage.[62] Interestingly, not only the extent but also the nature of RBC vesiculation mechanisms may vary with storage time,[60] in terms of size, structure,[62] protein composition,[60,62,85,99-103] or PS exposure.[85] In addition, microvesiculation exhibits dependence on RBC age,[66] as well as on manufacturing method and storage settings, including the presence of ASs, leukoreduction,[26,104] and the plasticizer material used in blood bags.[105] In particular, prestorage leukoreduction reduces the total levels of both RBC-derived macroparticles and microparticle-mediated procoagulant and inflammatory markers.[96,98,101]

Mass spectrometry–based proteomics studies of RBC membrane and vesicles suggested that vesiculation in vitro is a different process than vesiculation in vivo.[60,102] Indeed, in vivo vesiculation is suggested to be an integral part of the cellular homeostasis and physiologic aging process[106] for the efficient disposal of damaged or dangerous RBC components.[92] However, storage-related disturbances in cellular metabolism (energy depletion), biomechanical properties, calcium, ceramide, and PS exposure levels further exacerbate the formation of microparticles.[95,107,108] Immunoblotting,[62] flow cytometric,[103] and proteomic analysis[60,99-102] suggested that several factors may influence the vesicles release profile during storage, including the storage-dependent acceleration of RBC protein breakdown and oxidation, as described.

Clarifying the root causes of RBC vesiculation in vitro is critical for improvement of current blood component processing and storage strategies. For example, it has recently been reported that, in a murine model of transfusion, addition of antioxidants to stored RBCs units results in a significant decrease in microparticle formation as well as improved RBC 24-hour posttransfusion recovery and recipient alloimmunization.[109] Additional beneficial effects might derive from the introduction of filters designed to remove immunoglobulins, cytokines, and other bioactive proteins from aged RBC supernatants.[110]

MICRO RNAS

Micro RNAs (miRNAs) are known to be involved in posttranscriptional or translational control, which should be absent in anucleated ribosome-free RBCs. However, when assaying 52 miRNAs in stored RBCs, Kannan and Atreya[111] detected a significant alteration in the levels of miR-96, miR-150, miR-196a, and miR-197, which increased during the first 20 days of storage and decreased thereafter. These miRNAs might derive from residual white blood cells (WBCs) and PLTs in the unit. Indeed, modern prestorage leuko- and PLT-reduction filters only remove 3 to 3.5 and approximately 2 logs of WBCs and PLTs, respectively.[112] Nucleated WBCs and PLTs contain machinery to process pre-miRNAs into mature miRNAs, and specific PLT miRNA levels have been found to correlate with PLT reactivity.[113] Functional studies in the future will determine whether and to what extent miRNAs accumulating over storage might affect transfusion recipients.

POSTTRANSLATIONAL MODIFICATIONS

Although hitherto underinvestigated, protein posttranslational modifications might represent a key biologic mediator of molecular signaling events triggered by storage-dependent variables. For example, kinases such as AMPK and PKC might be activated by ATP consumption and intracellular calcium accumulation, and thus mediate downstream signaling. Phosphorylation of downstream targets might thus affect enzyme activities or the stability of structural proteins. Of significance, control of the RBC shape and membrane dynamics are both consequences of dynamic cytoskeleton alterations at spectrin junctions, a process requiring ATP hydrolysis.[114] This local remodeling of the membrane is likely related to ATP-driven phosphorylation of specific structural proteins, a reoccurring theme in the regulation of membrane stability. Indeed, coupling between the phospholipid bilayer and the spectrin-actin network governs the deformability of RBCs through complex protein–protein interactions that are modulated by phosphorylation.[115,116] Interestingly, recent studies have documented changes in the phosphorylation status of membrane proteins in sickle RBCs[117-119] and associated these events to morphology-related abnormalities typical of diseased RBCs. Similar studies on stored RBCs are still missing, although one of the hallmarks of storage lesions is the irreversible alteration of the shape phenotype, which is mediated by membrane perturbations and cytoskeleton dysfunctions, as discussed. By means of phosphoproteomics technologies, we recently observed a storage duration–dependent increase in the Ser/Thr phosphorylation status of some crucial RBC membrane proteins (e.g., AE1, spectrin, ankyrin, Band 4.1, and adducin).[120] In line with available models of the RBC membrane organization, these preliminary data confirm a pivotal role in the regulation of membrane mechanics and RBC surface remodeling of the 4.1R macrocomplex and the adducin-to-cytoskeleton bridged complex, which contains the membrane-spanning proteins AE1 and glycophorin C. The reduced survival of transfused RBCs might thus be attributable, in part, to deformability-linked phosphorylation events.[120]

Other posttranslational modifications, such as the above-discussed (non-)enzymatic glycosylation might also be deleterious to RBC function. For example, advanced glycation endproducts of proteins from stored

RBCs increase endothelial ROS generation through the interaction with the receptor for advanced glycation end products in the recipient.[121]

DONOR VARIABILITY AND PREANALYTICAL ISSUES

Sample heterogeneity is a critical issue in omics studies.[42] Variability in donated units arises from biologic (donor) and technical factors, also referred to as preanalytical issues.[122] These variables complicate in vitro studies of RBC storage, limit RBC storage system development, and confound studies aimed at determining the impact of storage lesions on transfusion outcomes.[123]

Variability in RBC storage characteristics among healthy donors is a long-recognized phenomenon that goes by the name of "storability." Storability has been a major unsolved problem throughout the history of blood banking. The variability issue was first recognized in the 1960s by Dern and colleagues[124] and remains a concern in modern storage strategies. Indeed, under the same conditions, different blood donors have markedly different RBC pre- and posttransfusion capacities. Unknown donor-related factors not only affect a range of physiologic properties of stored cells, but they have also been shown to represent the most significant contributing factors influencing in-bag hemolysis and RBC recovery.[91,124-126] A major factor affecting these variables is the donor-dependent RBC capacity to cope with oxidative injury, as gleaned through recent investigations on poststorage viability either in inbred mouse strains or small groups of humans.[125] Several RBC storage–dependent physiologic variables have been found to display donor dependence as well, including leukoreduction-associated hemolysis,[26] RBC age upon donation,[17,127] metabolic rate and metabolite concentrations (e.g., ATP),[128] fragility profiles,[129] membrane vesiculation degree,[26] susceptibility to oxidative stress,[53,130] and many more clinically significant properties of stored RBCs (such as vascular effects observed in the recipients).[131] Moreover, storage lesions appear to be dependent on donor sex, age, and smoking habit[132-134] and can be influenced by the genetic background of the donor. The genetic background either implies β-thalassemia traits, glucose-6-phosphate dehydrogenase deficiency,[135] intrinsic variation in RBC HbA1c,[136] or PS-exposure levels.[137]

Within this framework, the currently ongoing Recipient Epidemiology and Donor Evaluation Study (REDS)-III has been designed to test whether genetic characteristics of the donors underlie the interdonor variability observed in storage-related hemolysis.[138] Identifying the critical factors influencing storability may ease the assessment of donated blood quality and help tailor manufacturing strategies that could cope with the variability issue.

Other than donor variability, preanalytical issues[122] and processing strategies (leukofiltration, pathogen inactivation, ASs, rejuvenation, etc.)[139] influence the phenotype of RBCs. Examples of variability include supernatant K^+ levels and hemolysis,[140] inflammatory response mediators,[141] oxygen transport,[142] PS exposure,[137] eicosanoid mediators,[125] and the amount and composition of microparticles in the supernatant.[143] These variables are often a function of specific storage settings and manufacturing strategies.[139] Leukofiltration and ASs significantly alter biochemical profiles of RBC units, as gleaned through omics investigations.[8,26,101] Affected variables include the rate and extent of hemolysis, erythrophagocytosis, vesiculation, and oxidative stress management of the cells.[123,132,138,140] Finally, overnight hold of whole blood at room temperature before component processing affects several in vitro measures (ATP, 2,3-DPG, hemolysis)[144] and membrane properties (osmotic resilience, vesiculation).[145]

CONCLUSION

Transfusion of RBCs still represents one of the most valuable life-saving treatments in many areas of modern medicine. Despite controversial retrospective clinical studies, prospective evidence recommending against the use of RBC units stored longer than 2 weeks as an issuable blood-derived therapeutics is still missing or inconclusive. On the other hand, an accumulating body of evidence from biochemical, morphologic, and omics investigations suggests that RBCs stored longer than 14 days are characterized by the accumulation of a series of lesions that make them qualitatively different from fresh RBCs. As of now, it is unclear whether and to what extent these lesions might end up compromising the safety and effectiveness of the transfusion therapy.

However, the hereby reviewed literature can help pave the way for the development of alternative storage strategies aimed at abrogating the potential risk factors associated with the transfusion of older units. In this view, omics technologies can guide the development and testing of currently available or future alternatives to routine storage. Examples include, but are not limited to, the introduction and optimization of alternative storage strategies (cryostorage[146,147] or deoxygenation[148,149]) or (additive-rejuvenation[150]) solutions, such as those envisaging the implementation of alkaline pH[151] or antioxidants[109,152] in the storage unit. Additionally, future biochemical and omics studies should be applied to emerging technologies in the field of transfusion medicine, such as stem cell–derived ex vivo generated RBCs.[153]

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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ADA and AGK coordinated the joint efforts of the contributing authors; ADA, AGK, SR, and MHA wrote the paper and prepared the figures; KCH, LZ, and ISP critically commented on the paper and contributed to its finalization; and ADA and KCH revised the paper.

REFERENCES

1. Koch CG, Li L, Sessler DI, et al. Duration of red-cell storage and complications after cardiac surgery. N Engl J Med 2008;358:1229-39.
2. Lelubre C, Piagnerelli M, Vincent JL. Association between duration of storage of transfused red blood cells and morbidity and mortality in adult patients: myth or reality? Transfusion 2009;49:1384-94.
3. Cohen B, Matot I. Aged erythrocytes: a fine wine or sour grapes? Br J Anaesth 2013;111:i62-i70.
4. D'Alessandro A, Liumbruno G, Grazzini G, et al. Red blood cell storage: the story so far. Blood Transfus 2010;8: 82-8.
5. Lion N, Crettaz D, Rubin O, et al. Stored red blood cells: a changing universe waiting for its map(s). J Proteomics 2010;73:374-85.
6. Karon BS, Van Buskirk CM, Jaben EA, et al. Temporal sequence of major biochemical events during blood bank storage of packed red blood cells. Blood Transfus 2012;28: 1-9.
7. D'Alessandro A, Zolla L. Biochemistry of red cell aging in vivo and storage lesions. European Haematology Association—EHA 18 Educational Book. Haematologica 2013;98:389-96.
8. D'Alessandro A, D'Amici GM, Vaglio S, et al. Time-course investigation of SAGM-stored leukocyte-filtered red bood cell concentrates: from metabolism to proteomics. Haematologica 2012;97:107-15.
9. Blasi B, D'Alessandro A, Ramundo N, et al. Red blood cell storage and cell morphology. Transfus Med 2012;22:90-6.
10. Leidl K, Liebisch G, Richter D, et al. Mass spectrometric analysis of lipid species of human circulating blood cells. Biochim Biophys Acta 2008;1781:655-64.
11. Paglia G, Palsson BØ, Sigurjonsson OE. Systems biology of stored blood cells: can it help to extend the expiration date? J Proteomics 2012;76:163-7.
12. Bosman GJ, Lasonder E, Groenen-Döpp YA, et al. Comparative proteomics of erythrocyte aging in vivo and in vitro. J Proteomics 2010;73:396-402.
13. Bosman GJ, Werre JM, Willekens FL, et al. Erythrocyte ageing in vivo and in vitro: structural aspects and implications for transfusion. Transfus Med 2008;18:335-47.
14. Lang E, Qadri SM, Lang F. Killing me softly—suicidal erythrocyte death. Int J Biochem Cell Biol 2012;44:1236-43.
15. D'Alessandro A, Blasi B, D'Amici GM, et al. Red blood cell subpopulations in freshly drawn blood: application of proteomics and metabolomics to a decades-long biological issue. Blood Transfus 2013;11:1-13.
16. Ghashghaeinia M, Cluitmans JC, Akel A, et al. The impact of erythrocyte age on eryptosis. Br J Haematol 2012;157: 606-14.
17. Marrocco C, Pallotta V, D'Alessandro A, et al. Red blood cell populations and membrane levels of peroxiredoxin 2 as candidate biomarkers to reveal blood doping. Blood Transfus 2012;10:s71-7.
18. Mock DM, Widness JA, Veng-Pedersen P, et al. Measurement of post-transfusion red cell survival with the biotin label. Transfusion Med Rev 2014;pii: S0887-7963(14)00022-4.
19. Bennett-Guerrero E, Veldman TH, Doctor A, et al. Evolution of adverse changes in stored RBCs. Proc Natl Acad Sci U S A 2007;104:17063-8.
20. Gelderman MP, Yazer MH, Jia Y, et al. Serial oxygen equilibrium and dynamic measurements during RBC storage. Transfus Med 2010;20:341-5.
21. Gevi F, D'Alessandro A, Rinalducci S, et al. Alterations of red blood cell metabolome during cold liquid storage of erythrocyte concentrates in CPD-SAGM. J Proteomics 2012;76:168-80.
22. Romero PJ, Romero EA. Determinant factors for an apparent increase in oxygen affinity of senescent human erythrocytes. Acta Cient Venez 2004;55:83-5.
23. Burr MJ. The relationship between pH and aerobic glycolysis in human and canine erythrocytes. Comp Biochem Physiol B 1972;41:687-94.
24. Tsai AG, Hofmann A, Cabrales P, et al. Perfusion vs. oxygen delivery in transfusion with "fresh" and "old" red blood cells: the experimental evidence. Transfus Apher Sci 2010;43:69-78.
25. Wallas CH. Sodium and potassium changes in blood bank stored human erythrocytes. Transfusion 1979;19: 210-5.
26. Antonelou MH, Tzounakas VL, Velentzas AD, et al. Effects of pre-storage leukoreduction on stored red blood cells signaling: a time-course evaluation from shape to proteome. J Proteomics 2012;76:220-38.
27. Wiley JS, McCulloch KE, Bowden DS. Increased calcium permeability of cold-stored erythrocytes. Blood 1982;60: 92-8.
28. Pompeo G, Girasole M, Cricenti A, et al. Erythrocyte death in vitro induced by starvation in the absence of Ca(2+). Biochim Biophys Acta 2010;1798:1047-55.
29. Kurata M, Suzuki M. Glutathione regeneration in calcium-loaded erythrocytes: a possible relationship among calcium accumulation, ATP decrement and oxidative damage. Comp Biochem Physiol B Biochem Mol Biol 1994;109:305-12.
30. White PH, Plishker GA. Calcium-dependent association of glutathione S-transferase with the human erythrocyte membrane. Biochem Biophys Res Commun 1983;114:488-92.

31. Nakao K, Wada T, Kamiyama T, et al. A direct relationship between adenosine triphosphate-level and in vivo viability of erythrocytes. Nature 1962;194:877-8.
32. Valeri CR, Hirsch NM. Restoration in vivo of erythrocyte adenosine triphosphate, 2,3-diphosphoglycerate, potassium ion, and sodium ion concentrations following the transfusion of acid-citrate-dextrose-stored human red blood cells. J Lab Clin Med 1969;73:722-33.
33. Goodman SR, Daescu O, Kakhniashvili DG, et al. The proteomics and interactomics of human erythrocytes. Exp Biol Med (Maywood) 2013;238:509-18.
34. D'Alessandro A, Righetti PG, Zolla L. The red blood cell proteome and interactome: an update. J Proteome Res 2010;9:144-63.
35. D'Alessandro A, Zolla L. Proteomics and metabolomics in cancer drug development. Expert Rev Proteomics 2013;10: 473-88.
36. Messana I, Ferroni L, Misiti F, et al. Blood bank conditions and RBCs: the progressive loss of metabolic modulation. Transfusion 2000;40:353-60.
37. Pertinhez TA, Casali E, Lindner L, et al. Biochemical assessment of red blood cells during storage by 1H nuclear magnetic resonance spectroscopy. Identification of a biomarker of their level of protection against oxidative stress. Blood Transfus 2014;5:1-9.
38. Nishino T, Yachie-Kinoshita A, Hirayama A, et al. In silico modeling and metabolome analysis of long-stored erythrocytes to improve blood storage methods. J Biotechnol 2009;144:212-23.
39. Roback JD, Josephson CD, Waller EK, et al. Metabolomics of ADSOL (AS-1) red blood cell storage. Transfus Med Rev 2014;pii: S0887-7963(14)00004-2.
40. Nishino T, Yachie-Kinoshita A, Hirayama A, et al. Dynamic simulation and metabolome analysis of long-term erythrocyte storage in adenine-guanosine solution. PloS One 2013;8:e71060.
41. Burger P, Korsten H, De Korte D, et al. An improved red blood cell additive solution maintains 2,3-diphosphoglycerate and adenosine triphosphate levels by an enhancing effect on phosphofructokinase activity during cold storage. Transfusion 2010;50:2386-92.
42. Sparrow RL. Time to revisit red blood cell additive solutions and storage conditions: a role for "omics" analyses. Blood Transfus 2012;10:s7-11.
43. Whillier S, Raftos JE, Sparrow RL, et al. The effects of long-term storage of human red blood cells on the glutathione synthesis rate and steady-state concentration. Transfusion 2011;51:1450-9.
44. Kumar P, Maurya PK. L-cysteine efflux in erythrocytes as a function of human age: correlation with reduced glutathione and total anti-oxidant potential. Rejuvenation Res 2013;16:179-84.
45. Carrell RW, Winterbourn CC, Rachmilewitz EA. Activated oxygen and hemolysis. Br J Haematol 1975;30: 259-64.
46. D'Amici GM, Rinalducci S, Zolla L. Proteomic analysis of RBC membrane protein degradation during blood storage. J Proteome Res 2007;6: 3242-55.
47. Antonelou MH, Kriebardis AG, Stamoulis KE, et al. Red blood cell aging markers during storage in citrate-phosphate-dextrose-saline-adenine-glucose-mannitol. Transfusion 2010;50:376-89.
48. Lewis IA, Campanella ME, Markley JL, et al. Role of band 3 in regulating metabolic flux of red blood cells. Proc Natl Acad Sci U S A 2009;106:18515-20.
49. Castagnola M, Messana I, Sanna MT, et al. Oxygen-linked modulation of erythrocyte metabolism: state of the art. Blood Transfus 2010;8:53-8.
50. Pantaleo A, Ferru E, Giribaldi G, et al. Oxidized and poorly glycosylated band 3 is selectively phosphorylated by Syk kinase to form large membrane clusters in normal and G6PD-deficient red blood cells. Biochem J 2009;418: 359-67.
51. Matte A, Bertoldi M, Mohandas N, et al. Membrane association of peroxiredoxin-2 in red cells is mediated by the N-terminal cytoplasmic domain of band 3. Free Radic Biol Med 2012;55C:27-35.
52. Low FM, Hampton MB, Peskin AV, et al. Peroxiredoxin 2 functions as a noncatalytic scavenger of low-level hydrogen peroxide in the erythrocyte. Blood 2007;109: 2611-7.
53. Rinalducci S, D'Amici GM, Blasi B, et al. Peroxiredoxin-2 as a candidate biomarker to test oxidative stress levels of stored red blood cells under blood bank conditions. Transfusion 2011;51:1439-49.
54. Lutz HU, Bogdanova A. Mechanisms tagging senescent red blood cells for clearance in healthy humans. Front Physiol 2013;4:387.
55. Karon BS, Hoyer JD, Stubbs JR, et al. Changes in Band 3 oligomeric state precede cell membrane phospholipid loss during blood bank storage of red blood cells. Transfusion 2009;49:1435-42.
56. Rinalducci S, Ferru E, Blasi B, et al. Oxidative stress and caspase-mediated fragmentation of cytoplasmic domain of erythrocyte band 3 during blood storage. Blood Transfus 2012;10:s55-62.
57. Kriebardis AG, Antonelou MH, Stamoulis KE, et al. Storage-dependent remodeling of the red blood cell membrane is associated with increased immunoglobulin G binding, lipid raft rearrangement, and caspase activation. Transfusion 2007;47:1212-20.
58. Walpurgis K, Kohler M, Thomas A, et al. Storage-induced changes of the cytosolic red blood cell proteome analyzed by 2D DIGE and high-resolution/high-accuracy MS. Proteomics 2012;12:3263-72.
59. D'Amici GM, Mirasole C, D'Alessandro A, et al. Red blood cell storage in SAGM and AS3: a comparison through the membrane two-dimensional electrophoresis proteome. Blood Transfus 2012;10:46-54.

60. Bosman GJ, Lasonder E, Luten M, et al. The proteome of red cell membranes and vesicles during storage in blood bank conditions. Transfusion 2008;48:827-35.
61. Kriebardis AG, Antonelou MH, Stamoulis KE, et al. Progressive oxidation of cytoskeletal proteins and accumulation of denatured hemoglobin in stored red cells. J Cell Mol Med 2007;11:148-55.
62. Kriebardis AG, Antonelou MH, Stamoulis KE, et al. RBC-derived vesicles during storage: ultrastructure, protein composition, oxidation, and signaling components. Transfusion 2008;48:1943-53.
63. Delobel J, Prudent M, Rubin O, et al. Subcellular fractionation of stored red blood cells reveals a compartment-based protein carbonylation evolution. J Proteomics 2012; 76:181-93.
64. Kriebardis AG, Antonelou MH, Stamoulis KE, et al. Membrane protein carbonylation in non-leukodepleted CPDA-preserved red blood cells. Blood Cells Mol Dis 2006;36: 279-82.
65. D'Alessandro A, Mirasole C, Zolla L. Haemoglobin glycation (Hb1Ac) increases during red blood cell storage: a MALDI-TOF mass-spectrometry-based investigation. Vox Sang 2013;105:177-80.
66. Sparrow RL, Veale MF, Healey G, et al. Red blood cell (RBC) age at collection and storage influences RBC membrane-associated carbohydrates and lectin binding. Transfusion 2007;47:966-8.
67. Geng Q, Romero J, Saini V, et al. Extracellular 20S proteasomes accumulate in packed red blood cell units. Vox Sang 2009;97:273-4.
68. Corsi D, Paiardini M, Crinelli R, et al. Alteration of α-spectrin ubiquitination due to age-dependent changes in the erythrocytes membrane. Eur J Biochem 1999;261: 775-83.
69. Burger P, Hilarius-Stokman P, de Korte D, et al. CD47 functions as a molecular switch for erythrocyte phagocytosis. Blood 2012;119:5512-21.
70. Neelam S, Kakhniashvili DG, Wilkens S, et al. Functional 20S proteasomes in mature human red blood cells. Exp Biol Med (Maywood) 2011;236:580-91.
71. Ellmerer M, Pachler C, Plank J. Tight glycemic control in the hospital. J Diabetes Sci Technol. 2008;2: 728-31.
72. Dumaswala UJ, Zhuo L, Jacobsen DW, et al. Protein and lipid oxidation of banked human erythrocytes: role of glutathione. Free Radic Biol Med 1999;27: 1041-9.
73. Virgili F, Battistini N, Canali R, et al. High glucose-induced membrane lipid peroxidation on intact erythrocytes and on isolated erythrocyte membrane (ghosts). J Nutr Biochem 1996;7:151-61.
74. Silliman CC, Moore EE, Kelher MR, et al. Identification of lipids that accumulate during the routine storage of prestorage leukoreduced red blood cells and cause acute lung injury. Transfusion 2011;51:2549-54.
75. Baumgartner JM, Nydam TL, Clarke JH, et al. Red blood cell supernatant potentiates LPS-induced proinflammatory cytokine response from peripheral blood mononuclear cells. J Interferon Cytokine Res 2009; 29:333-8.
76. Pittman JG, Martin DB. Fatty acid biosynthesis in human erythrocytes: evidence in mature erythrocytes for an incomplete long chain fatty acid synthesizing system. J Clin Invest 1966;45:165-72.
77. Bosman GJ, Cluitmans JC, Groenen YA, et al. Susceptibility to hyperosmotic stress-induced phosphatidylserine exposure increases during red blood cell storage. Transfusion 2011;51:1072-8.
78. Timperio AM, Mirasole C, D'Alessandro A, et al. Red blood cell lipidomics analysis through HPLC-ESI-qTOF: application to red blood cell storage. J Integr Omics 2013; 3:11-24.
79. Bicalho B, Holovati JL, Acker JP. Phospholipidomics reveals differences in glycerophosphoserine profiles of hypothermically stored red blood cells and microvesicles. Biochim Biophys Acta 2013;1828:317-26.
80. Dinkla S, Wessels K, Verdurmen WP, et al. Functional consequences of sphingomyelinase-induced changes in erythrocyte membrane structure. Cell Death Dis 2012; 3:e410.
81. Mohandas N, Chasis JA. Red blood cell deformability, membrane material properties and shape: regulation by transmembrane, skeletal and cytosolic proteins and lipids. Semin Hematol 1993;30:171-92.
82. Arduini A, Minetti G, Ciana A, et al. Cellular properties of human erythrocytes preserved in saline-adenineglucose-mannitol in the presence of L-carnitine. Am J Hematol 2007;82:31-40.
83. Berezina TL, Zaets SB, Morgan C, et al. Influence of storage on red blood cell rheological properties. J Surg Res 2002;102:6-12.
84. Sens P, Gov N. Force balance and membrane shedding at the red-blood-cell surface. Phys Rev Lett 2007;98:018102.
85. Almizraq R, Tchir JD, Holovati JL, et al. Storage of red blood cells affects membrane composition, microvesiculation, and in vitro quality. Transfusion 2013; 53:2258-67.
86. Barshtein G, Guralb A, Noga M, et al. Storage-induced damage to red blood cell mechanical properties can be only partially reversed by rejuvenation. Transfus Med Hemother 2014;41:197-204.
87. Henkelman S, Dijkstra-Tiekstra MJ, de Wildt-Eggen J. Is red blood cell rheology preserved during routine blood bank storage? Transfusion 2010;50:941-8.
88. Karger R, Lukow C, Kretschmer V. Deformability of red blood cells and correlation with ATP content during storage as leukocyte-depleted whole blood. Transfus Med Hemother 2012;39:277-82.
89. Hess JR. Measures of stored red blood cell quality. Vox Sang 2014;107:1-9.

90. Safeukui I, Buffet PA, Deplaine G, et al. Quantitative assessment of sensing and sequestration of spherocytic erythrocytes by human spleen: implications for understanding clinical variability of membrane disorders. Blood 2012;120:424-30.
91. Bosman GL. Survival of red blood cells after transfusion: processes and consequences. Front Physiol 2013;4:376.
92. Willekens FL, Werre JM, Groenen-Döpp YA, et al. Erythrocyte vesiculation: a self-protective mechanism? Br J Haematol 2008;141:549-56.
93. Greenwalt TJ. The how and why of exocytic vesicles. Transfusion 2006;46:143-52.
94. Donadee C, Raat NJ, Kanias T, et al. Nitric oxide scavenging by red blood cell microparticles and cell-free hemoglobin as a mechanism for the red cell storage lesion. Circulation 2011;124:465-U294.
95. Simak J, Gelderman MP. Cell membrane microparticles in blood and blood products: potentially pathogenic agents and diagnostic markers. Transfus Med Rev 2006;20: 1-26.
96. Jy W, Ricci M, Shariatmadar S, et al. Microparticles in stored red blood cells as potential mediators of transfusion complications. Transfusion 2011;51:886-93.
97. Kim-Shapiro DB, Lee J, Gladwin MT. Storage lesion: role of red blood cell breakdown. Transfusion 2011;51: 844-51.
98. Gao Y, Lv L, Liu S, et al. Elevated levels of thrombin-generating microparticles in stored red blood cells. Vox Sang 2013;105:11-7.
99. Tissot JD, Canellini G, Rubin O, et al. Blood microvesicles: from proteomics to physiology. Transl Proteomics 2013;1: 38-52.
100. Anniss AM, Glenister KM, Killian JJ, et al. Proteomic analysis of supernatants of stored red blood cell products. Transfusion 2005;45:1426-33.
101. Dzieciatkowska M, Silliman CC, Moore EE, et al. Proteomic analysis of the supernatant of red blood cell units: the effects of storage and leucoreduction. Vox Sang 2013;105:210-8.
102. Bosman GJ, Lasonder E, Groenen-Döpp YA, et al. The proteome of erythrocyte-derived microparticles from plasma: new clues for erythrocyte aging and vesiculation. J Proteomics 2012;76:203-10.
103. Canellini G, Rubin O, Delobel J, et al. Red blood cell microparticles and blood group antigens: an analysis by flow cytometry. Blood Transfus 2012;10:s39-45.
104. Sparrow RL, Sran A, Haeley G, et al. In vitro measures of membrane changes reveal differences between red blood cells stored in saline-adenineglucose-mannitol and AS-1 additive solutions: a paired study. Transfusion 2013;54: 560-8.
105. Dumont LJ, Baker S, Dumont DF, et al. Exploratory in vitro study of red blood cell storage containers formulated with an alternative plasticizer. Transfusion 2012;52: 1439-45.
106. Bosman GJ, Willekens FL, Werre JM. Erythrocyte aging: a more than superficial resemblance to apoptosis? Cell Physiol Biochem 2005;16:1-8.
107. Palek J, Stewart G, Lionetti FJ. The dependence of shape of human erythrocyte ghosts on calcium, magnesium and adenosine triphosphate. Blood 1974;44:583- 97.
108. Huang YX, Wu ZJ, Mehrishi J, et al. Human red blood cell aging: correlative changes in surface charge and cell properties. J Cell Mol Med 2011;15:2634-42.
109. Stowell SR, Smith NH, Zimring JC, et al. Addition of ascorbic acid solution to stored murine red blood cells increases posttransfusion recovery and decreases microparticles and alloimmunization. Transfusion 2013; 53:2248-57.
110. Sowemimo-Coker SO. Evaluation of an experimental filter designed for improving the quality of red blood cells (RBCs) during storage by simultaneously removing white blood cells and immunomodulators and improving RBC viscoelasticity and Band 3 proteins. Transfusion 2014;54: 592-601.
111. Kannan M, Atreya C. Differential profiling of human red blood cells during storage for 52 selected microRNAs. Transfusion 2010;50:1581-8.
112. Silliman CC, Kelher MR, Khan SY, et al. Experimental prestorage filtration removes antibodies and decreases lipids in RBC supernatants mitigating TRALI in vivo. Blood 2014;123:3488-95.
113. Edelstein LC, McKenzie SE, Shaw C, et al. MicroRNAs in platelet production and activation. J Thromb Haemost 2013;1:340-50.
114. Park Y, Best CA, Auth T, et al. Metabolic remodeling of the human red blood cell membrane. Proc Natl Acad Sci U S A 2010;107:1289-94.
115. Manno S, Takakuwa Y, Nagao K, et al. Modulation of erythrocyte membrane mechanical function by protein 4.1 phosphorylation. J Biol Chem 2005;280:7581-7.
116. Manno S, Takakuwa Y, Nagao K, et al. Modulation of erythrocyte membrane mechanical function by beta-spectrin phosphorylation and dephosphorylation. J Biol Chem 1995;270:5659-65.
117. Siciliano A, Turrini F, Bertoldi M, et al. Deoxygenation affects tyrosine phosphoproteome of red cell membrane from patients with sickle cell disease. Blood Cells Mol Dis 2010;44:233-42.
118. George A, Pushkaran S, Li L, et al. Altered phosphorylation of cytoskeleton proteins in sickle red blood cells: the role of protein kinase C, Rac GTPases, and reactive oxygen species. Blood Cells Mol Dis 2010;45: 41-5.
119. Soderblom EJ, Thompson JW, Schwartz EA, et al. Proteomic analysis of ERK1/2-mediated human sickle red blood cell membrane protein phosphorylation. Clin Proteomics 2013;10:1.
120. Longo V, Marrocco C, Zolla L, et al. Label-free quantitation of phosphopeptide changes in erythrocyte

membranes: towards molecular mechanisms underlying deformability alterations in stored red blood cells. Haematologica 2014;99:e122-5.
121. Mangalmurti NS, Chatterjee S, Cheng G, et al. Advanced glycation end products on stored red blood cells increase endothelial reactive oxygen species generation through interaction with receptor for advanced glycation end products. Transfusion 2010;50:2353-61.
122. Delobel J, Rubin O, Prudent M, et al. Biomarker analysis of stored blood products: emphasis on pre-analytical issues. Int J Mol Sci 2010;11:4601-17.
123. Chassé M, English SW, McIntyre L, et al. Effect of blood donor characteristics on transfusion outcomes: a protocol for systematic review and meta-analysis. Syst Rev 2014;3: 28.
124. Dern RJ, Gwinn RP, Wiorkowski JJ. Studies on the preservation of human blood. I. Variability in erythrocyte storage characteristics among healthy donors. J Lab Clin Med 1966;67:955-65.
125. Zimring JC, Smith N, Stowell SR, et al. Strain-specific red blood cell storage, metabolism, and eicosanoid generation in a mouse model. Transfusion 2014;54:137-48.
126. Hess JR, Sparrow RL, van der Meer PF, et al. Biomedical Excellence for Safer Transfusion (BEST) Collaborative. Red blood cell hemolysis during blood bank storage: using national quality management data to answer basic scientific questions. Transfusion 2009;49:2599-603.
127. Greenwalt TJ, Dumaswala UJ. Effect of red cell age on vesiculation in vitro. Br J Haematol 1988;68:465-7.
128. van't Erve TJ, Wagner BA, Martin SM, et al. The heritability of metabolite concentrations in stored human red blood cells. Transfusion 2014;54:2055-63.
129. Tarasev M, Alfano K, Chakraborty S, et al. Evaluation of novel in-vitro RBC fragility metrics as age-independent measures of stored RBC quality. Transfusion 2011;51:79A.
130. Kanias T, Gladwin MT. Nitric oxide, hemolysis, and the red blood cell storage lesion: interactions between transfusion, donor, and recipient. Transfusion 2012;52: 1388-92.
131. Roback JD. Vascular effects of the red blood cell storage lesion. Hematology Am Soc Hematol Educ Program 2011; 2011:475-9.
132. Antonelou MH, Kriebardis AG, Stamoulis KE, et al. Apolipoprotein J/clusterin in human erythrocytes is involved in the molecular process of defected material disposal during vesiculation. PloS One 2011;6:e26033.
133. Norton JM, Rand PW. Decreased deformability of erythrocytes from smokers. Blood 1981;57:671-4.
134. Raval JS, Waters JH, Seltsam A, et al. Menopausal status affects the susceptibility of stored RBCs to mechanical stress. Vox Sang 2011;100:418-21.
135. Francis RO, Jhang JS, Pham HP, et al. Glucose-6-phosphate dehydrogenase deficiency in transfusion medicine: the unknown risks. Vox Sang 2013;105: 271-82.
136. Wenk RE, McGann H, Gibble J. Haemoglobin A1c in donor erythrocytes. Transfus Med 2011;21:349-50.
137. Dinkla S, Peppelman M, Van Der Raadt J, et al. Phosphatidylserine exposure on stored red blood cells as a parameter for donor-dependent variation in product quality. Blood Transfus 2013;3:1-6.
138. Kleinman S, Busch MP, Murphy EL, et al. The National Heart, Lung, and Blood Institute Recipient Epidemiology and Donor Evaluation Study (REDS-III): a research program striving to improve blood donor and transfusion recipient outcomes. Transfusion 2014;54:942-55.
139. Wagner SJ, Glynn SA, Welniak LA, et al. Research opportunities in optimizing storage of red blood cell products. Transfusion 2013;54:483-94.
140. McAteer MJ, Dumont LJ, Cancelas J, et al. Multi-institutional randomized control study of haemolysis in stored red cell units prepared manually or by an automated system. Vox Sang 2010;99:34-43.
141. Dean MM, Samson LD, Rooks K, et al. Donor variation in biological mediators during storage of packed red blood cells. Blood 2013;122:3655.
142. Buchwald H, Menchaca HJ, Michalek VN, et al. Pilot study of oxygen transport rate of banked red blood cells. Vox Sang 2009;96:44-8.
143. Bastos-Amador P, Royo F, Gonzalez E, et al. Proteomic analysis of microvesicles from plasma of healthy donors reveals high individual variability. J Proteomics 2012;75: 3574-384.
144. van der Meer PF, Cancelas JA, Cardigan R, et al. BEST Collaborative. Evaluation of overnight hold of whole blood at room temperature before component processing: effect of red blood cell (RBC) additive solutions on in vitro RBC measures. Transfusion 2011;51:15S-24S.
145. Veale MF, Healey G, Sparrow RL. Effect of additive solutions on red blood cell (RBC) membrane properties of stored RBCs prepared from whole blood held for 24 hours at room temperature. Transfusion 2011;51:25S-33S.
146. Pallotta V, D'Amici GM, D'Alessandro A, et al. Red blood cell processing for cryopreservation: from fresh blood to deglycerolization. Blood Cells Mol Dis 2012;48:226-32.
147. Holovati JL, Wong KA, Webster JM, et al. The effects of cryopreservation on red blood cell microvesiculation, phosphatidylserine externalization, and CD47 expression. Transfusion 2008;48:1658-68.
148. D'Alessandro A, Gevi F, Zolla L. Red blood cell metabolism under prolonged anaerobic storage. Mol Biosyst 2013;9:1196-209.
149. Yoshida T, Shevkoplyas SS. Anaerobic storage of red blood cells. Blood Transfus 2010;8:220-36.
150. Meyer EK, Dumont DF, Baker S, et al. Rejuvenation capacity of red blood cells in additive solutions over long-term storage. Transfusion 2011;51:1574-9.
151. Hess JR, Hill HR, Oliver CK, et al. Alkaline CPD and the preservation of red blood cell 2,3-DPG. Transfusion 2002; 42:747-52.

152. Pallotta V, Gevi F, D'Alessandro A, et al. Red blood cell storage with vitamin C and N-acetylcysteine prevents oxidative stress-related lesions: a metabolomics overview. Blood Transfus 2014;12:367-87.

153. Hricik T, Federici G, Zeuner A, et al. Transcriptomic and phospho-proteomic analyzes of erythroblasts expanded in vitro from normal donors and from patients with polycythemia vera. Am J Hematol 2013;88:723-72.