Editorial

"Valar morghulis": all red cells must die

Hara T. Georgatzakou[1], Marianna H. Antonelou[2], Effie G. Papageorgiou[1], Anastasios G. Kriebardis[1*]

[1]*Laboratory of Reliability and Quality Control in Laboratory Hematology (HemQcR), Department of Biomedical Sciences, School of Health & Caring Sciences, University of West Attica (UniWA), Egaleo;*
[2]*Department of Biology, School of Science, National & Kapodistrian University of Athens (NKUA), Athens, Greece*

Correspondence: Anastasios G. Kriebardis
e-mail: akrieb@uniwa.gr

Eryptosis is the programmed cell death of red blood cells (RBCs) in response to different kinds of stress. Several hallmarks of eryptosis have been determined over the years, including increased calcium influx, phosphatidylserine (PS) externalisation, cell shrinkage, and membrane vesiculation[1]. Even though medically-induced eryptosis (such as the interventional experiments reported in this issue or during RBC aging under blood bank conditions) may differ compared to the aging processes *in vivo*, some molecular signalling pathways are thought to be common[2]. As such, the study of stored RBCs under various physiological cell stressors could offer mechanistic insight into the life and death of RBCs *in vivo*, in health and disease. At the same time, metabolomics constitutes an advanced research tool to perform high throughput analysis of energy metabolism in RBCs.

In the present issue of Blood Transfusion, Nemkov *et al.*[3], already well established in the field of metabolomics, especially in transfusion medicine, and having the experience and expertise to define the critical steps of metabolic pathways in RBCs under various environmental changes[4-7], provide for the first time information on metabolic flows and shifts in RBCs exposed to different eryptosis-inducing stimuli, mimicking common physiological cellular stresses.

Through state-of-the-art mass spectrometry-based approaches the authors showed that ionomycin-induced ionic stress promoted increases in purine oxidation and fatty acid mobilisation, but decreased glycolysis. Of note, those metabolic changes were consistent with profiles usually observed in RBCs stored under blood bank conditions[4,6,8]. In contrast, hyperosmotic stress increased glycolysis and glutathione synthesis. This finding could drive the development of novel hyperosmotic blood storage solutions to promote glycolysis and glutathione synthesis that are progressively inhibited by storage, leading to oxidative lesions and RBC death[9]. The authors also showed that glucose starvation led to the deregulation of glutathione (GSH) synthesis, increased externalisation of PS and accumulation of cytosolic fatty acids; these findings are tentatively suggestive of a Ca^{2+}-independent phospholipase activity in RBCs, an interesting hypothesis that, if confirmed, might expand our understanding of the already complicated cascade of events that regulate RBC lipid metabolism. However, glucose starvation combined with the absence of Ca^{2+} resulted in activation of the pentose phosphate pathway, probably as a mechanism targeting the equilibrium in NADH and NADPH reducing equivalents[4]. In addition, the authors showed that heat stress negatively impacts glycolysis and ATP

Blood Transfus 2020; 18: 83-5 DOI 10.2450/2020.0028-20

production, which in turn accelerates GSH synthesis and turnover (an ATP-dependent process), ultimately causing increase in intracellular calcium. All these changes were associated with a high pyruvate/lactate ratio, a finding that is consistent with an overactivation of NADH-dependent methemoglobin reductase. Finally, another important finding of this paper was the accumulation of choline in the supernatant of starved RBCs. Choline release by those stressed RBCs is indicative of an active protein damage-repair pathway, some intermediate metabolites of which may participate in vascular tone response to stress.

A reduced lifespan of eryptotic cells has been encountered in a multitude of pathological conditions, including chronic renal failure, hepatic failure, sepsis, malignancies, diabetes, etc.[10]. Under these pathological conditions, RBCs are subjected to hyperosmotic or ionic stress (e.g., by uremic toxin accumulation), hyperthermia and starvation (e.g., by fever in sepsis and hypoglycaemia in renal or hepatic failure). Even though eryptosis has long been shown in these pathological states[11-13], the metabolic impact of eryptotic processes on RBCs was totally elusive. Consequently, application of metabolomics can give some explanation for the disturbances in morphology, redox potential and other functional characteristics commonly observed in those clinical conditions[14,15]. The ability of RBC to respond immediately to environmental changes through glucose metabolism, the sole main source of energy for these cells, defines their ultimate fate in the circulation. The metabolomic profiles of cells responding to various types of physiological stressors *in vivo* probably include life or death biomarkers critically involved in their functions, recognition, and clearance.

Besides respiratory homeostasis, RBCs contribute to the regulation of the redox potential of blood, of the immune system and of vascular tone[16-18]. Modifications in specific metabolites imposed by certain disease states or drugs may enhance or block those critical regulatory functions of RBCs. Consequently, studies like the present one can provide significant insights into metabolic characteristics of RBCs intrinsically linked to their capacity to function properly under stresses and stimulators found in a variety of common diseases. Failure to cope with such stresses could result in anaemia due to premature destruction of RBCs.

However, regardless of the RBC ability to respond to different types of stress, their fate is, after all, predetermined. This means that, sooner or later, all RBCs will inevitably die, but their ability to respond successfully to stress determines when precisely this final event will happen. Ultimately, while all RBCs must die ("Valar Morghulis"; "all men must die", to borrow from the Game of Thrones" universe of George RR Martin) before time comes, they all must serve their physiological purpose ("Valar Dohaeris"; "all men must serve", in the acclaimed "Song of Ice and Fire" series). As they struggle to stay alive and do their job till the last moment, they must adapt to the unfriendly environment elicited by environmental stressors in the face of many common diseases. The elucidation of the metabolomic arm of strategies evolved in RBCs to cope with various stressors *in vivo* from a metabolic standpoint can provide clues as to how to boost them in the face of physiological, pathological or medically-imposed challenges (e.g., blood storage).

The Authors declare no conflicts of interest.

REFERENCES

1. Lang F, Qadri SM. Mechanisms and significance of eryptosis, the suicidal death of erythrocytes. Blood Purif 2012; **33**: 125-30.
2. Antonelou MH, Kriebardis AG, Papassideri IS. Aging and death signalling in mature red cells: from basic science to transfusion practice. Blood Transfus 2010; **8** (Suppl 3): s39-47.
3. Nemkov T, Qadri SM, Sheffield WP, D'Alessandro A. Decoding the metabolic landscape of pathophysiologic stress-induced cell death in anucleate red blood cells, Blood Transfus 2020; **18**: 129-44.
4. Nemkov T, Sun K, Reisz JA, et al. Hypoxia modulates the purine salvage pathway and decreases red blood cell and supernatant levels of hypoxanthine during refrigerated storage. Haematologica 2018; **103**: 361-72.
5. Reisz JA, Wither MJ, Dzieciatkowska M, et al. Oxidative modifications of glyceraldehyde 3-phosphate dehydrogenase regulate metabolic reprogramming of stored red blood cells. Blood 2016; **128**: e32-42.
6. D'Alessandro A, Culp-Hill R, Reisz JA, et al. Heterogeneity of blood processing and storage additives in different centers impacts stored red blood cell metabolism as much as storage time: lessons from REDS-III-Omics. Transfusion 2019; **59**: 89-100.
7. Nemkov T, Reisz JA, Xia Y, et al. Red blood cells as an organ? How deep omics characterization of the most abundant cell in the human body highlights other systemic metabolic functions beyond oxygen transport. Expert Rev Proteomics 2018; **15**: 855-64.
8. Howie HL, Hay AM, de Wolski K, et al. Differences in Steap3 expression are a mechanism of genetic variation of RBC storage and oxidative damage in mice. Blood Adv 2019; **3**: 2272-85.
9. D'Alessandro A, Kriebardis AG, Rinalducci S, et al. An update on red blood cell storage lesions, as gleaned through biochemistry and omics technologies. Transfusion 2015; **55**: 205-19.
10. Qadri SM, Bissinger R, Solh Z, Oldenborg PA. Eryptosis in health and disease: a paradigm shift towards understanding the (patho) physiological implications of programmed cell death of erythrocytes. Blood Rev 2017; **31**: 349-61.
11. Lang F, Bissinger R, Abed M, Artunc F. Eryptosis - the neglected cause of anemia in end stage renal disease. Kidney Blood Press Res 2017; **42**: 749-60.
12. Kempe DS, Akel A, Lang PA, et al. Suicidal erythrocyte death in sepsis. J Mol Med (Berl) 2007; **85**: 273-81.
13. Bissinger R, Lang E, Gonzalez-Menendez I, et al. Genetic deficiency of the tumor suppressor protein p53 influences erythrocyte survival. Apoptosis 2018; **23**: 641-50.
14. Georgatzakou HT, Antonelou MH, Papassideri IS, Kriebardis AG. Red blood cell abnormalities and the pathogenesis of anemia in end-stage renal disease. Proteomics Clin Appl 2016; **10**: 778-90.
15. Baskurt OK, Gelmont D, Meiselman HJ. Red blood cell deformability in sepsis. Am J Respir Crit Care Med 1998; **157**: 421-7.
16. Buttari B, Profumo E, Rigano R. Crosstalk between red blood cells and the immune system and its impact on atherosclerosis. Biomed Res Int 2015; **2015**: 616834.
17. Byrnes JR, Wolberg AS. Red blood cells in thrombosis. Blood 2017; **130**: 1795-9.
18. Buehler PW, Alayash AI. Redox biology of blood revisited: the role of red blood cells in maintaining circulatory reductive capacity. Antioxid Redox Signal 2005; **7**: 1755-60.