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EDITORIAL

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The Wnt (Wingless related-integration site)-signaling pathway has been implicated in the maintenance of a variety of developmental processes, including cell differentiation, growth, and motility.[1–3] The developmental role of Wnt signaling has been characterized primarily in stem and progenitor cell populations and is associated with canonical β-catenin mediated transcriptional regulation in nucleated cells.[2,4–6] In this volume of *ATVB*, Siman-Tov et al[7] characterized a novel role of Wnt signaling in enucleated erythrocytes and uncovered that regulation of the red blood cell (RBC) cytoskeleton and erythrocyte survival was controlled by noncanonical Wnt signals.[7] Erythrocytes undergo tremendous strain when traveling through pressurized capillaries where gas exchange with tissues can occur.[8,9] The elastic properties of the erythrocyte cytoskeleton permit RBC survival in this strenuous microenvironment, traversing vessels of multiple diameters, flow rates, and pressures.[9] If the cytoskeleton cannot accommodate this elasticity, hemolysis can result and contribute to anemias or other blood disorders.[8]

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Siman-Tov et al[7] hypothesized that treatment of erythrocytes with Wnt ligands would improve RBC survival while allowing for the maintenance of an adaptable morphology. Indeed, erythrocytes have been previously shown to contain JNK (c-Jun N-terminal kinase), Rac1 (Ras-related C3 botulinum toxic substrate 1), RhoA (Ras homolog family member A), and PKC (protein kinase C), all integral components associated with the noncanonical Wnt pathway. Specifically, RAC GTPases, stimulated by PKC activation, physically increased actin polymerization by elongating preexisting filaments or by synthesizing new actin from globular actin stores.[7] Thus, noncanonical Wnt signaling induced actin polymerization, a key regulator in maintaining the shape and survival of erythrocytes.[7]

In a series of elegant molecular experiments, Siman-Tov et al[7] revealed a significant improvement in erythrocyte survival and function when RBC was incubated with Wnt5, a unique ligand involved primarily in the activation of noncanonical Wnt pathways (Figure). Enhancements in erythrocyte morphology were demonstrated by increased levels of filamentous actin, greater hemoglobin concentration, and enlarged mean corpuscle volume. To determine whether these beneficial cell morphologies could increase oxygen-carrying capacity in mature erythrocytes, the group transfused Wnt-activated RBC into C57BL/6J mice.[7] Compared with control, transfused RBC pretreated with Wnt5 demonstrated increased erythrocyte survival at 7-day postinjection with improved oxygen delivery while maintaining normal cell morphology.[7] Thus, stimulation of circulating RBC with Wnt5 resulted in a significant improvement in the capacity for erythrocytes to perfuse ischemic tissues and deliver oxygen systemically.

Next, Siman-Tov et al[7] investigated how Wnt activation ex vivo would affect the performance of cryopreserved erythrocytes.[7] Typically, RBCs are stored in CPDA (citrate phosphate dextrose-adenine)-1 media. However, this storage buffer routinely increases the risk

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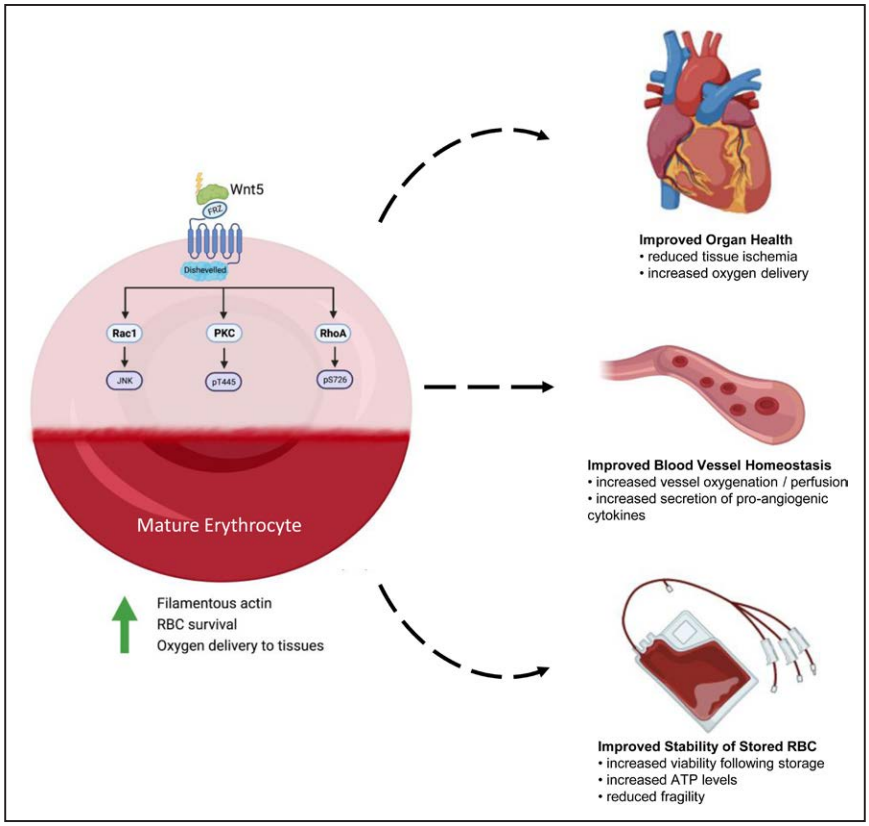

Figure. Clinical implications for the activation of noncanonical WNT (Wingless related-integration site) signals in red blood cell (RBC) proposed by Siman-Tov et al.[7] Following activation of the noncanonical Wnt pathway by Wnt5, increased expression of proteins RAC1 (Ras-related C3 botulinum toxic substrate 1), RhoA (Ras homolog family member A), and PKC (protein kinase C) stimulate actin polymerization in mature erythrocytes. This modification in the RBC cytoskeleton augmented erythrocyte survival and increased oxygen saturation within the peripheral circulation. The clinical applications of Wnt5 to enhance erythrocyte health include (1) improved oxygen delivery and perfusion, (2) reduced tissue ischemia, (3) improved blood vessel homeostasis and repair, (4) improved stability of cryopreserved RBC, and (5) increased erythrocyte survival after cryopreservation and transfusion. Created with BioRender.com.

of lesions in the cell membrane that lead to osmotic fragility and impaired in oxygen transport. Specifically, stored RBC demonstrated reduced oxygen affinity, increased adhesions to endothelial cells, and increased risk of bacterial contamination.[10] As such, transfusion of previously cryopreserved blood into recipients has the potential to introduce damage that elevates the risk of deleterious reactions to blood products. Interestingly, erythrocytes cryopreserved with Wnt5 demonstrated increased viability following storage in both PBS and CPDA-1, highlighting a potential role for Wnt supplementation to better preserve RBCs for storage. Testing of next generation, pathway-specific synthetic Wnt ligands is now warranted during blood product cryopreservation and delivery.[10]

It is well established that the preservation erythrocyte function is extremely important in the maintenance of blood vessel homeostasis.[11,12] Erythrocytes carry oxygen throughout systemic vasculature for delivery to tissues. This often-overlooked function is required when attempting to activate endogenous mechanisms to mitigate hypoxia and tissue ischemia. Indeed, atherosclerosis contributes to ischemic cardiovascular disease that can culminate in myocardial infarction and stroke.[13] Ischemic cardiovascular disease is responsible for ≈9 million deaths globally and 126 million cases per year.[14] Thus, the development of translational techniques to improve oxygen delivery after surgery may prove instrumental in reducing the global incidence of ischemic comorbidities.

Another significant risk factor for the development of cardiovascular disease is the presence of high blood pressure or hypertension.[14] Hypertension is estimated to affect ≈50% of US adults according to the National Health and Nutrition Examination Survey, in 2018.[15] As such, use of antihypertensive agents has become increasingly prevalent in clinical practice to limit the adverse effects of chronic hypertension on blood vessel health. Uncovering mechanisms through which erythrocytes can effectively deliver oxygen under the added stress of high blood pressure would represent a significant step towards improved cardiovascular outcomes. The ability of noncanonical Wnt-pathway activation to modulate RBC cytoskeleton adaptability while maintaining cellular volume provides a mechanism through which erythrocytes can navigate high-pressured vessels while still delivering oxygen throughout the peripheral circulation.

Additionally, the increased prevalence of atherosclerosis and hypertension in patients with metabolic disease such as obesity and type 2 diabetes brings with it challenges associated with tissue perfusion.[16] Indeed, the ability of the Wnt pathway to increase RBC flexibility may prove essential in combatting hypoxia by ensuring that RBCs better traverse occluded and inflamed vessels and capillaries. Perhaps increased oxygen delivery through improved erythrocyte health may slow the development of unstable plaque formation or thrombotic release and reduce the risk of myocardial infarction or stroke in patients with advanced atherosclerosis.

Manipulation of the Wnt pathway provides immediate benefits not only for disease manifestation but for the ex vivo storage and transfusion of RBC. In individuals receiving emergent care or major surgery, blood loss remains a major complication.[17,18] The demand for cryopreserved blood and plasma products to ensure adequate perfusion is increasingly high. As mentioned by Siman-Tov et al,[7] there are increased hazards associated with the preservation and delivery of blood that impact cardiovascular health, including reduced oxygen affinity of RBCs. After treatment with Wnt5, however, there were significant reductions in RBC damage following irradiation and transfusion.

Finally, this article discusses the possible application of augmenting Wnt5-mediated RBC signaling in situ. Siman-Tov established that monocytes and lymphocytes represent a vital source for increased release of Wnt5.[7] Monocytes give rise to macrophages that stimulate arteriogenesis, a form of blood vessel remodeling characterized by collateral vessel widening and increased perfusion.[19–21] Understanding the implications of Wnt5 signaling and RBC survival in the growth and maintenance of healthy vasculature represents an essential endogenous mechanism used to avert capillary occlusion and may prove beneficial in mitigating tissue ischemia in individuals with peripheral and coronary artery disease. Thus, the realization of active Wnt-signaling axis in the circulation may prove to be a novel signaling paradigm during arteriogenic, angiogenic, and vasculogenic processes represents an exciting future direction for this work.

These seminal experiments by Siman-Tov et al[7] demonstrate a profound improvement in enucleated erythrocyte homeostasis through the stimulation of the noncanonical Wnt pathway. This advancement may provide another piece of the puzzle for the development of improved strategies to preserve cardiovascular health.

ARTICLE INFORMATION

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Disclosures

None.

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