

Review

Matrix Mechanosensation in the Erythroid and Megakaryocytic Lineages

Christina M. Ward ¹ and Katya Ravid ^{1,2,*}

¹ Department of Medicine and Whitaker Cardiovascular Institute, Boston University School of Medicine, Boston, MA 02118, USA; cmward@bu.edu

² Departments of Biochemistry and Biology, Boston University School of Medicine, Boston, MA 02118, USA

* Correspondence: kravid@bu.edu

Received: 11 March 2020; Accepted: 2 April 2020; Published: 6 April 2020



Abstract: The biomechanical properties of the bone marrow microenvironment emerge from a combination of interactions between various extracellular matrix (ECM) structural proteins and soluble factors. Matrix stiffness directs stem cell fate, and both bone marrow stromal and hematopoietic cells respond to biophysical cues. Within the bone marrow, the megakaryoblasts and erythroblasts are thought to originate from a common progenitor, giving rise to fully mature megakaryocytes (the platelet precursors) and erythrocytes. Erythroid and megakaryocytic progenitors sense and respond to the ECM through cell surface adhesion receptors such as integrins and mechanosensitive ion channels. While hematopoietic stem progenitor cells remain quiescent on stiffer ECM substrates, the maturation of the erythroid and megakaryocytic lineages occurs on softer ECM substrates. This review surveys the major matrix structural proteins that contribute to the overall biomechanical tone of the bone marrow, as well as key integrins and mechanosensitive ion channels identified as ECM sensors in context of megakaryocytosis or erythropoiesis.

Keywords: extracellular matrix; megakaryocyte; erythroid lineage; integrins; mechanosensitive ion channels

1. Introduction

1.1. Overview

The bone marrow (BM) is the predominate location for adult hematopoiesis—the process in which a population of multipotent hematopoietic stem cells (HSCs) produce lineage-restricted progenitors that give rise to all the types of blood cells. The BM microenvironment is complex and supports the proliferation and differentiation of hematopoietic stem progenitor cells (HSPCs) through cues received from the extracellular matrix (ECM). The ECM is a non-cellular support network composed of structural proteins and various soluble factors [1,2]. As the physical support structure for the surrounding HSPCs, the ECM has a role in biological functions, such as adhesion, migration, apoptosis, proliferation, and differentiation [3]. The composition of the ECM is derived from a mixture of collagens; laminins; fibronectin and fibrinogen; and soluble proteins, such as cytokines, chemokines, and secreted enzymes. These various structural matrix proteins set the elasticity and rigidity of the bone marrow that creates the biophysical state surrounding the cells [1–3]. The ability of a cell to sense forces such as compression, tension, fluid shear stress, and hydrostatic pressure within the three-dimensional environment is a conserved ability that all single celled organisms and complex multicellular eukaryotes possess [3]. Characterizing the functional link from the biomechanical cues a cell receives to a biochemical response is the process called mechanotransduction [3]. This review focuses on the biomechanical properties of the BM compartment and how these properties influence the cell fate of the erythroid and megakaryocyte (MK) lineages through the process of mechanotransduction.

1.2. Bone Marrow ECM Composition

BM stromal and hematopoietic progenitor cells have been demonstrated to be mechanically responsive to engineered substrates and surrounding viscosity [4–8]. The rigidity of the BM is not uniform throughout, as there exists a large amount of heterogeneity in the Young's modulus ranging from 0.25 to 24.7 kPa [9]. Several key matrix structural proteins contribute to the overall biomechanical tone of the bone marrow. The ECM is mainly made up of collagens, which include both fibrillary collagen (collagen I, II, III, V, and XI) and non-fibrillary collagen [10]. Immunohistochemical analysis of bone marrow revealed that collagen I and IV along with fibronectin are localized throughout the endosteum [11]. Multicolor quantitative confocal imaging cytometry, a technique that has allowed for a three-dimensional map of the ECM components, further confirmed that collagen I and fibronectin are pervasively localized throughout the entire bone marrow [12]. Furthermore, collagen IV, laminin, and fibronectin are largely present in the BM parenchyma [12]. This agrees with other sources that have also mapped type IV collagen, laminin, and fibronectin to the sinusoids [13]. The presence of collagen throughout the bone marrow contributes to the overall stiffness of the ECM [1,10]. The increase in stiffness is non-linear in response to increased deposition of collagen III mixed with collagen I [14,15]. Fibronectin, a glycoprotein, modulates ECM stiffness by organizing collagen into fibrils [16]. A collagen matrix failed to develop in fibronectin-deficient mouse fibroblasts that were cultured, but the addition of exogenous fibronectin reconstituted the collagen matrix [16].

The BM ECM is continuously subjected to remodeling by proteins such as matrix metalloproteinases (MMP), tissue inhibitors of MMPs (TIMPs), and plasmins. MMPs are a family of zinc-dependent endopeptidases which are responsible for the breakdown of the ECM, while TIMPs counterbalance the function of MMPs [17]. In patients with essential thrombocythemia and polycythemia vera plasma levels of MMP-3 were decreased and plasma TIMP-1 was elevated [18]. These data suggest that altered BM ECM composition contributes to these disease states. Endosteal expression of TIMP-3 promotes HSC regeneration and actively drives HSCs out of the quiescent state [19,20]. This is consistent with other studies that demonstrate that ECM substrate stiffness that resembles the endosteal space promotes HSC proliferation [8]. Plasmin is a protein that breaks down ECM fibrin, fibronectin, and laminin, and activates MMPs [21]. Urokinase plasminogen activator (uPA), which is broadly expressed by BM cells, activates plasmin [21–23]. Active plasmin is regulated by plasminogen activator inhibitor 1 (PAI-1) and tissue plasminogen activator (tPA) [21]. In the BM the expressions of PAI-1 and tPA offset one another functionally and are important for hematopoietic regeneration, particularly after BM transplantation [24,25]. Inhibition of PAI-1 improved hematopoietic recovery in mice after myeloablation. Taken together, the structural protein makeup of the BM ECM and the factors regulating these proteins are influential on the overall rigidity of the BM.

1.3. Effect of BM Stiffness on Cell Differentiation

ECM stiffness is important to the determination of HSC fate and differentiation [4,8,26]. HSCs cultured on stiffer matrices promoted the development of myeloid progenitors, while softer laminin surfaces increased the number of erythroid precursors [8]. Rigidity influences BM stromal cell differentiation [27]. Osteogenic differentiation of BM-derived mesenchymal stromal/stem cells (MSCs) is enhanced on stiffer substrate surfaces [27]. ECM stiffness modulates MK development and platelet production. When BM progenitors were cultured in 3D methylcellulose hydrogels designed to recapitulate the BM microenvironment (30–60 Pa), MKs had more demarcation membranes, higher ploidy, and produced more platelets compared to liquid cultures or stiffer 3D cultures (300–600 Pa) [28]. This was further corroborated by another study, wherein MKs were cultured on soft collagen-coated gels (300 Pa) or on stiffer gels (34 kPa) [29]. These cultured MKs had higher ploidy on the softer gels regardless of collagen concentration [29]. In fact, matrix stiffness seems to mediate MK differentiation and platelet production independent of the type of collagen. In an ex vivo model, cultured MKs produced more platelets on softer matrices regardless of whether the matrix was

composed of collagen I or collagen IV [30,31]. $\beta 1$ integrin was more highly activated on the softer matrix, and platelet formation on the softer silk was mediated through the PI3K/Akt pathway [30].

2. Integrins as ECM Sensors in the Context of Erythropoiesis

Integrins are heterodimeric type I transmembrane proteins composed of α and β subunits and serve as one of the major cell adhesion receptors in the ECM. Integrins act as a transmembrane link between the ECM and the cytoskeleton. The 24 mammalian integrin receptors are made up of a combination of 18 α and 8 β subunits [32]. Each subunit has a single transmembrane domain, a short cytoplasmic tail, and an extracellular domain that binds ligands such as collagen or fibronectin [32]. Integrins on the surface of blood cells and platelets are inactive in a bent conformation, and activation is triggered by variety of signaling molecules such as growth factors [32]. Integrin-ECM binding activates a multitude of intracellular pathways involved in fundamental biological processes, such as cell survival and proliferation, cytoskeletal organization, and cell mobility [32]. Knockout of integrin $\beta 1$ in mice is lethal [33]. However, $\alpha 2$ integrin knockout mice are viable and develop normally, but platelet aggregation in these mice is delayed [34]. Integrin $\alpha 4$ is required to maintain normal hematopoiesis [35], and lack of $\alpha 4$ integrin disrupts T-cell and B-cell production, whereas monocyte and natural killer cell production remains unaffected by $\alpha 2$ knockout [36]. Mutations in integrin $\beta 3$ lead to the disorder Glanzmann's thrombasthenia, wherein platelets cannot bind fibrinogen and fail to aggregate [37]. $\beta 3$ mutations contribute to cardiovascular disease and the development of atherosclerosis [37]. Integrins are essential for mechanosensing of the ECM and related signaling.

Erythropoiesis, the process in which progenitors undergo terminal differentiation into mature circulating red blood cells (RBCs), occurs in a specialized bone marrow structure called the erythroblastic island, which is characterized by a central macrophage surrounded by committed erythroid progenitors. Integrins are crucial to this process as they not only facilitate a connection to the surrounding ECM but mediate cell to cell contact between the central macrophage and erythroid progenitors. The predominate integrins expressed on erythroid progenitors are VLA-4 ($\alpha 4\beta 1$) and VLA-5 ($\alpha 5\beta 1$); both are fibronectin receptors [38]. While erythroid maturation is mediated considerably through growth factor signaling, principally through erythropoietin (EPO), adhesion to extracellular fibronectin and laminin promotes erythroid development [8,38]. The developmental expression of $\alpha 4$ can be used as a helpful tool to identify erythroid cells at different maturation states, as $\alpha 4$ expression decreases as the erythroid progenitors mature into terminally differentiated reticulocytes cells [39–41]. The decreased ability of erythroid cells to bind fibronectin correlates with the expression pattern of $\alpha 4$ during development; with early human erythroid progenitors (CFU-E) adhering to fibronectin, the intermediate progenitor (proerythroblast) has a diminished capability to bind to fibronectin, and terminal differentiated reticulocytes are unable to bind fibronectin [39]. This is necessary so that mature RBCs can exit the bone marrow and enter circulation. The effects of erythroid adhesion to fibronectin through integrins are temporally distinct as early *ex vivo* fetal liver erythroid progenitors require stimulation with erythropoietin (EPO), but fibronectin was found to be crucial for differentiation of late progenitors [38]. Erythroid progenitors' engagement with fibronectin can be perturbed with the use of monoclonal antibodies against VLA-5 [39]. Inhibiting VLA-4 engagement with fibronectin blocked erythroid development; however, blocking VLA-5 in the same fashion did not have the same effect [42]. The roles of the two predominate fibronectin integrins on erythroid progenitors are debated, as it has not always been clear from *in vivo* models what distinct functions these fibronectin-binding integrins serve [43–45]. This raises the possibility that the stiffness of fibronectin used in the different studies could influence the effect of these integrins on erythroid differentiation. Several integrin knockout mice were generated and administered phenyl hydrazine which induces severe anemia and stress erythropoiesis. The integrin, $\beta 1$, which partners with $\alpha 4$ or $\alpha 5$ is required for splenic erythropoiesis under the stress, but bone marrow stress erythropoiesis produces progenitor cells that are able to mobilize to the peripheral blood, although the late progenitors have maturation defects [45]. In the mouse model wherein integrin $\alpha 4$ is deleted, the mice experience a delayed recovery of the erythroid

progenitor population in response to phenyl hydrazine and defective erythroid cells under both basal and stressed conditions [45]. When further knockout mouse models were created in which the integrins $\alpha 4$ or $\alpha 5$ were selectively removed in the erythroid lineage conditionally either early or late in erythroid development, only $\alpha 4$ was required for proper erythroid maturation [44]. Collectively, this work demonstrates the importance of adhesion to the ECM via integrins.

3. Integrins as ECM Sensors in the Context of Megakaryocytosis

MKs also conduct forces across the membrane through integrin attachment to ECM proteins. Since collagen has such a large role in ECM biomechanical properties, it has been the focus of research for the understanding of MK development and platelet formation [30,46,47]. MKs express two main receptors for collagen, integrin- $\alpha 2\beta 1$ and glycoprotein VI [47]; however, MK expression of leukocyte-associated immunoglobulin-like 1 (LAIR1) and discoidin domain receptor (DDR1) that also bind collagen have been described in the literature [48,49]. Early MK progenitors (CFU-MK) have a lower affinity for adhering to collagen compared to later MK progenitors [50], which is interesting as integrin- $\alpha 2\beta 1$ and glycoprotein VI expression occurs early in MK development [50,51]. It has been established that collagen I inhibits proplatelet formation (PPF) in order to prevent the release of immature platelets in the bone marrow, while collagen IV and laminin support PPF in the sinusoids [52]. To date, collagen I, III, and IV localization to a specific compartment of the bone marrow is elusive; MKs localize equally with collagen I and IV but more so with collagen IV within the vascular niche [51]. Studies utilizing antibodies and knockout mice implicate the inhibitory effects of collagen I on MK PPF by the glycoprotein VI, although there is a modest compensatory effect through integrin $\alpha 2\beta 1$ [51]. In studies where ex vivo human HSPC were co-cultured with human osteoblasts (hOST), MK maturation and PPF was inhibited [46]. This effect was mediated by the fibrillar structure of collagen I and engagement through integrin $\alpha 2\beta 1$ [46], and consequently activation of the Rho/Rock pathway [53]. MKs cultured on N-acetylated collagen I, which decreased mechanical tension, produced more platelets compared to unaltered collagen I [54]. This further supports the fact that the rigidity of the ECM in combination with the structural proteins affects platelet biogenesis [30,31]. Unlike collagen I, collagen III and IV promote platelet formation via the PI3K/Akt pathway [13,30]. Furthermore, primary MKs are able to self-regulate their immediate microenvironment by secreting ECM components collagen IV; fibronectin; laminin [13]; and lysyl oxidase (LOX), a copper-dependent enzyme, which controls ECM stiffness by regulating the cross-linking of collagen fibers [55]. Highly expressed in immature MKs and down-regulated in MKs of higher ploidy [56], LOX inhibition lessens BM fibrosis in the GATA-1^{low} and JAK2V671F mouse models [56,57].

The ECM component fibronectin plays a pivotal role in MK biology as it is necessary for platelet production [58], and when cultured CD34⁺ cells stimulated with thrombopoietin (TPO) were grown on a fibronectin matrix, the number of CFU-MK and percentage of CD41⁺ cells were higher compared to liquid only cultures [59]. HSPC cultures on fibronectin resulted in an increase in mouse HSC with an increase in MK output [13]. The major fibronectin binding integrins on MKs are VLA-4 ($\alpha 4\beta 1$) and VLA-5 ($\alpha 5\beta 1$), which are expressed throughout MK development [50,60]. HSC cultures produced more CFU-MKs on fibronectin as stiffness increased [8]. Integrin function on MKs is necessary, as a pan-blockade of integrins blocked TPO-stimulated growth; specifically neutralizing VLA-4 with monoclonal antibody blocked MK growth in TPO-stimulated bone marrow cultures [61]. In the MK cell line CHRF-288, full MK differentiation, CD41 expression, adhesion, size, polyploidy, and PPF were only achieved on cultures with fibronectin [58]. Integrins transmit mechanical signals via engagement with the ECM; these are relayed into the cells by activation of focal adhesion kinase (FAK) which subsequently activates the RhoA/ROCK pathway [62]. Activation of the RhoA/Rock mobilizes the cytoskeleton in MKs by phosphorylation of non-muscle myosin II (NMM-II) which is inhibitory to PPF [63]. Taken together, studies show that outcomes of specific integrin binding/activation depend on the matrix as well.

4. Mechanosensitive Ion Channels in the Erythroid Lineage

Mechanosensitive ion channels are pore forming channels that specialize in sensing physical sensations, such as force, osmotic pressure, stretching, or shear stress, and converting the stimuli into biochemical signals through the transfer of ions across the cellular membrane [64]. There are multiple mechanosensitive channel types, such as transient receptor potential cation channels, mechanosensitive potassium channels, and Piezo ion channels [64]. These channel families differ in their structure, function, activation, and cation selectivity [64]. This review focuses on the mechanosensitive ion channels in the TRP and Piezo families, as relatively few studies to date address mechanosensitive ion channels in the context of erythroid and MK biology. In the family of mechanically activated channels known as the transient receptor potential cation channel subfamily, member 3 and 6 (TRPC3 and TRPC6) are Ca^{2+} permeable non-selective ion channels that are downstream of PLC and DAG [65]. Both TRPC3 and TRPC6 are agonist activated [65,66]. Transient receptor potential vanilloid 4 (TRPV4) also is a Ca^{2+} permeable non-selection ion channel that functions in the capacity of an osmosensor, thermosensor, and like TRPC3 and TRPC6 can be activated by agonist stimulation [67–72]. The Piezo channel family has no sequence homology to any other mechanosensitive channel family [73]. The Piezo family of proteins was identified by neuroscientist Ardem Patapoutian who named two related ion channel proteins Piezo1 and Piezo2 – from the Greek word piezein, meaning squeeze/press. Piezo family channels activate in response to indentation and stress [64], and then the influx of Ca^{2+} into the cell triggers a series of signaling events that propagate the mechanical signal.

Two members of the family of mechanically activated channels, transient receptor potential cation channel subfamily, TRPC3 and TRPC6, are present on the surfaces of human erythroid progenitors and mature RBCs, allowing the passage of calcium (Ca^{2+}) through the membrane [74–76]. Erythroid cells and specifically RBCs are very sensitive to small changes in intracellular ion concentrations. Stimulation of erythroid TRPC3 increased intracellular Ca^{2+} in an EPO dependent manner [76,77] and is negatively modulated by TRPC6 [77]. TRPC3 mobilizes to the plasma membrane after EPO stimulation [74] possibly through TRPC3 interaction with proteins involved in the cytoskeleton [78,79]. The function of TRPC6 is unaffected by EPO stimulation, and in the mature RBC activation may be dependent on osmotic changes [75]. TRPC6 may mediate eryptosis a mechanism to clear damaged RBCs [75]. Recent reports have contested whether the family of TRP channels can directly conduct mechanical sensation, and have suggested that activation of these channels is downstream of other mechanosensitive sensors [80].

Autosomal dominant hemolytic anemia hereditary xerocytosis is linked to gain of function mutations in the mechanosensitive ion channel Piezo1 [81,82]. Piezo1 is a broadly expressed mechanosensitive ion channel responsible for transforming mechanical stimuli such as touch, pressure, shear stress, and membrane tension into a biochemical signal through the transmembrane flow of Ca^{2+} across a number of cell types that include vascular epithelial and neuronal tissue. [73,83]. In RBCs, Piezo1 transmit stimuli related to membrane stretch, [84] distortion, [85], and curvature [86]. The Piezo1 channel is non-selectively pharmacologically blocked by the spider venom inhibitor, GsMTx4 [87]. GsMTx4 intercalates into membrane lipids and alters membrane fluidity, thereby altering membrane tension and altering Piezo1 activation kinetics [87]. Activation of Piezo1 either by stretch or stimulation by Yoda-1, a Piezo1 specific agonist [88], results in robust Ca^{2+} entry [89,90] and eventual dehydration of RBCs which is similar to the phenotype seen in RBCs with gain of function Piezo1 mutations [90]. Mechanistically, the increased intracellular Ca^{2+} opens the Gardos channel (KCa3.1), which controls RBC volume [91]. Gardos channel mutations result in hereditary xerocytosis as well [92,93]. Piezo1 expression is up-regulated during the erythroid differentiation of cultured CD34^+ HSPCs [82]. Yoda-1 activation of Piezo1 in ex vivo cultures alters the dynamics of normal erythroid differentiation by decreasing glycophorin A by 75%, altering the GATA2/GATA1 ratio, and reducing hemoglobinization [94]. Similar dynamics were observed in erythroid cells cultured from CD34^+ HSPCs obtained from individuals with gain of function Piezo1 mutations [94]. These studies

shed light on the functional characterization of the Piezo1 channel, where on the erythroid progenitor it functions to influence differentiation, but Piezo1 functions on the mature RBC to control cell volume.

5. Mechanosensitive Ion Channels in the Megakaryocyte Lineage

As discussed above, both the erythroid and megakaryocytic lineages detect and react to mechanical forces through integrins as well as mechanosensitive ion channels. Extracellular Ca^{2+} plays an important role in MK ability to sense the ECM, as MKs and/or MK cell lines (Meg-01) have been observed to express the mechanosensitive ion channels [30,95,96]. The mechanosensitive receptor TRPC6 has been linked to Ca^{2+} influx and platelet function [97–99]. Treatment of proliferating MK with 5 μM SKF96365, a TRPC6 specific inhibitor, reduced MK numbers by 50% [95]. Although linked to MK proliferation in vitro, knockout of TRPC6 in mice had no effect on platelet function either in vitro or in vivo [100]. The platelet response to activating agonists and aggregation studies conducted in vitro with platelets obtained from TRPC6 $-/-$ mice were not significantly different than those of wild-type mice [100]. Thrombus formation after mechanical injury of the abdominal aorta was also unaffected, as were tail-bleed times for wild-type and knockout mice [100]. In MKs, the mechanosensitive ion channel, TRPV4, senses soft ECM which increases the influx of intracellular Ca^{2+} , which leads to the activation of $\beta 1$ -integrin and Akt phosphorylation and increases platelet production [30]. Platelet production decreased with inhibition of TRPV4 on MKs cultured on collagen IV, and moreover, mice treated with LOX inhibitor (BAPN) had softer ECMs, higher platelet counts, and an increase in TRPV4 activity [30].

Piezo1 expression was found on platelets and the MK cell line, Meg-01 [96]. Piezo1 was activated in platelets and Meg-01 by fluid shear stress, and inhibition of Piezo1 with GsMTx-4 abrogated the Ca^{2+} influx [96]. Yoda-1 stimulation of platelets increased Ca^{2+} influx by over 170% [96]. In vitro thrombus formation of whole blood was reduced by GsMTx-4; however, platelet aggregation was unaffected [96]. As noted above, the role of Piezo1 function has been well-characterized in RBC physiology; Piezo1 activation by Yoda-1 alters erythroid differentiation kinetics [86,90,94]. Since erythrocytes and MKs share a common progenitor, Piezo1 may contribute to MK maturation, a role that needs to be clarified. Taken together these studies demonstrate that mechanosensitive ion channels present on the surface of MKs sense matrix rigidity and that the biomechanical tone of the ECM contributes the dynamics of MK maturation and platelet production.

6. Discussion

Both MKs and erythrocytes emerge from a common progenitor. Differential expression of integrins and ion channel mechanosensors in the fully differentiated cells of these lineages, as well as the ECM composition, impact their properties and courses of development, as further summarized in Table 1. In erythroid progenitors, adhesion to extracellular fibronectin by the integrins VLA-4 and VLA-5 plays a role in the dynamics of RBC production. Engagement of integrin- $\alpha 2\beta 1$ and/or glycoprotein VI with collagen I compared to collagen IV influences proplatelet formation by MKs.

MK progenitors require fibronectin for platelet formation which is mediated through integrins VLA-4 and VLA-5. The mechanosensitive ion channels of the family of mechanically activated channels known as the transient receptor potential channels of the canonical type, TRPC3 and TRPC6, are present on the surfaces of erythroid progenitors and mature RBCs and mediate EPO stimulated transmembrane Ca^{2+} influx. Erythroid expression of the mechanosensitive ion channel, Piezo1, plays a role erythroid progenitor proliferation and regulates RBC volume in response mechanical pressure. The functions of mechanosensitive ion channels, TRPC6, TRPV4, and Piezo1, on MKs and platelet biology, are starting to be elucidated. Taken together, the effects of bone marrow components and the biophysical state of the bone contribute to erythroid and MK lineage development, and a greater understanding of these interactions could shed more light on mechanisms of underlying diseases.

Table 1. Erythroid and megakaryocyte mechanosensors.

Mechanosensor	Comments	Reference
VLA-4 ($\alpha 4\beta 1$)	Expression and attachment to fibronectin decreases as erythroid progenitors differentiate	[39]
	Delay in recovery from stress erythropoiesis and defective erythroid cells found in $\alpha 4$ knockout mice	[45] [61]
	Enhances TPO stimulated MK growth	
VLA-5 ($\alpha 5\beta 1$)	Redundant role in erythroid development	[43,44]
$\beta 1$ -integrin	Required for stress induced splenic erythropoiesis	[45]
$\alpha 2\beta 1$	Engagement with collagen I inhibits Mk maturation and PPF	[46]
	Binding to collagen IV promotes MK maturation and PPF	[30]
Glycoprotein VI	Signals the inhibitory effect of collagen I on PPF in MKs	[51]
TRPC3	EPO dependent increase in Ca^{2+} influx in erythroid cells	[74,76,77]
TRPC6	Negatively modulates TRPC3 activity in erythroid cell	[77]
	Potentially mediates eryptosis	[75]
	Ca^{2+} entry into platelet; platelet activation Inhibition decreased the number of MKs in vitro	[97–99] [95]
TRPV4	Increases MK maturation and platelet formation on soft matrix, activate $\beta 1$ -integrin and PI3K/Akt pathway in MKs	[30]
Piezo1	Gain of function mutations cause Hereditary Xerocytosis	[81,82]
	Modulates RBC volume	[89,90]
	Role in erythroid differentiation; activation delays maturation	[94]
	Activated on platelets in response to shear stress; inhibition reduced in vitro thrombus formation	[96]

Author Contributions: C.M.W. and K.R. participated in writing, reviewing, and editing the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by NIH NHLBI grant R01HL136363 to K.R. C.M.W. was supported by NIH NHLBI Research Training grants in Biology and Research in Blood Diseases and Resources (T32 HL007501) awarded to Dr. Martin Steinberg, and Multidisciplinary Training in Cardiovascular Research (T32 HL007224) awarded to Dr. Naomi Hamburg and Dr. Richard Wainford.

Acknowledgments: We regret potential omissions of published studies considering the space limit.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Bonnans, C.; Chou, J.; Werb, Z. Remodelling the extracellular matrix in development and disease. *Nat. Rev. Mol. Cell Biol.* **2014**, *15*, 786–801. [[CrossRef](#)] [[PubMed](#)]
- Gattazzo, F.; Urciuolo, A.; Bonaldo, P. Extracellular matrix: A dynamic microenvironment for stem cell niche. *Biochim. Biophys. Acta* **2014**, *1840*, 2506–2519. [[CrossRef](#)] [[PubMed](#)]
- DuFort, C.C.; Paszek, M.J.; Weaver, V.M. Balancing forces: Architectural control of mechanotransduction. *Nat. Rev. Mol. Cell Biol.* **2011**, *12*, 308–319. [[CrossRef](#)] [[PubMed](#)]
- Engler, A.J.; Sen, S.; Sweeney, H.L.; Discher, D.E. Matrix elasticity directs stem cell lineage specification. *Cell* **2006**, *126*, 677–689. [[CrossRef](#)]
- Lee, J.; Abdeen, A.A.; Kilian, K.A. Rewiring mesenchymal stem cell lineage specification by switching the biophysical microenvironment. *Sci. Rep.* **2014**, *4*, 5188. [[CrossRef](#)]
- Sikavitsas, V.I.; Bancroft, G.N.; Holtorf, H.L.; Jansen, J.A.; Mikos, A.G. Mineralized matrix deposition by marrow stromal osteoblasts in 3D perfusion culture increases with increasing fluid shear forces. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 14683–14688. [[CrossRef](#)]
- Yang, C.; Tibbitt, M.W.; Basta, L.; Anseth, K.S. Mechanical memory and dosing influence stem cell fate. *Nat. Mater.* **2014**, *13*, 645–652. [[CrossRef](#)]

8. Choi, J.S.; Harley, B.A. Marrow-inspired matrix cues rapidly affect early fate decisions of hematopoietic stem and progenitor cells. *Sci. Adv.* **2017**, *3*, e1600455. [[CrossRef](#)]
9. Jansen, L.E.; Birch, N.P.; Schiffman, J.D.; Crosby, A.J.; Peyton, S.R. Mechanics of intact bone marrow. *J. Mech. Behav. Biomed. Mater.* **2015**, *50*, 299–307. [[CrossRef](#)]
10. Kolacna, L.; Bakesova, J.; Varga, F.; Kostakova, E.; Planka, L.; Necas, A.; Lukas, D.; Amler, E.; Pelouch, V. Biochemical and biophysical aspects of collagen nanostructure in the extracellular matrix. *Physiol. Res.* **2007**, *56*, S51–S60.
11. Nilsson, S.K.; Debatis, M.E.; Dooner, M.S.; Madri, J.A.; Quesenberry, P.J.; Becker, P.S. Immunofluorescence characterization of key extracellular matrix proteins in murine bone marrow in situ. *J. Histochem. Cytochem.* **1998**, *46*, 371–377. [[CrossRef](#)] [[PubMed](#)]
12. Coutu, D.L.; Kokkaliaris, K.D.; Kunz, L.; Schroeder, T. Three-dimensional map of nonhematopoietic bone and bone-marrow cells and molecules. *Nat. Biotechnol.* **2017**, *35*, 1202–1210. [[CrossRef](#)] [[PubMed](#)]
13. Malara, A.; Currao, M.; Gruppi, C.; Celesti, G.; Viarengo, G.; Buracchi, C.; Laghi, L.; Kaplan, D.L.; Balduini, A. Megakaryocytes contribute to the bone marrow-matrix environment by expressing fibronectin, type IV collagen, and laminin. *Stem Cells* **2014**, *32*, 926–937. [[CrossRef](#)] [[PubMed](#)]
14. Asgari, M.; Latifi, N.; Heris, H.K.; Vali, H.; Mongeau, L. In vitro fibrillogenesis of tropocollagen type III in collagen type I affects its relative fibrillar topology and mechanics. *Sci. Rep.* **2017**, *7*, 1392. [[CrossRef](#)]
15. Licup, A.J.; Munster, S.; Sharma, A.; Sheinman, M.; Jawerth, L.M.; Fabry, B.; Weitz, D.A.; MacKintosh, F.C. Stress controls the mechanics of collagen networks. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 9573–9578. [[CrossRef](#)]
16. Velling, T.; Risteli, J.; Wennerberg, K.; Mosher, D.F.; Johansson, S. Polymerization of type I and III collagens is dependent on fibronectin and enhanced by integrins alpha 11beta 1 and alpha 2beta 1. *J. Biol. Chem.* **2002**, *277*, 37377–37381. [[CrossRef](#)]
17. Page-McCaw, A.; Ewald, A.J.; Werb, Z. Matrix metalloproteinases and the regulation of tissue remodelling. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 221–233. [[CrossRef](#)]
18. Wang, J.C.; Novetsky, A.; Chen, C.; Novetsky, A.D. Plasma matrix metalloproteinase and tissue inhibitor of metalloproteinase in patients with agnogenic myeloid metaplasia or idiopathic primary myelofibrosis. *Br. J. Haematol.* **2002**, *119*, 709–712. [[CrossRef](#)]
19. Shen, Y.; Winkler, I.G.; Barbier, V.; Sims, N.A.; Hendy, J.; Levesque, J.P. Tissue inhibitor of metalloproteinase-3 (TIMP-3) regulates hematopoiesis and bone formation in vivo. *PLoS ONE* **2010**, *5*. [[CrossRef](#)]
20. Nakajima, H.; Ito, M.; Smookler, D.S.; Shibata, F.; Fukuchi, Y.; Morikawa, Y.; Ikeda, Y.; Arai, F.; Suda, T.; Khokha, R.; et al. TIMP-3 recruits quiescent hematopoietic stem cells into active cell cycle and expands multipotent progenitor pool. *Blood* **2010**, *116*, 4474–4482. [[CrossRef](#)]
21. Smith, H.W.; Marshall, C.J. Regulation of cell signalling by uPAR. *Nat. Rev. Mol. Cell Biol.* **2010**, *11*, 23–36. [[CrossRef](#)] [[PubMed](#)]
22. Hoyer-Hansen, G.; Behrendt, N.; Ploug, M.; Dano, K.; Preissner, K.T. The intact urokinase receptor is required for efficient vitronectin binding: Receptor cleavage prevents ligand interaction. *FEBS Lett* **1997**, *420*, 79–85. [[CrossRef](#)]
23. Li, H.; Daculsi, R.; Bareille, R.; Bourget, C.; Amedee, J. uPA and MMP-2 were involved in self-assembled network formation in a two dimensional co-culture model of bone marrow stromal cells and endothelial cells. *J. Cell Biochem.* **2013**, *114*, 650–657. [[CrossRef](#)] [[PubMed](#)]
24. Ibrahim, A.A.; Yahata, T.; Onizuka, M.; Dan, T.; Van Ypersele De Strihou, C.; Miyata, T.; Ando, K. Inhibition of plasminogen activator inhibitor type-1 activity enhances rapid and sustainable hematopoietic regeneration. *Stem Cells* **2014**, *32*, 946–958. [[CrossRef](#)]
25. McWilliam, N.A.; Robbie, L.A.; Barelle, C.J.; Adey, G.; Prasad, S.; Bennett, B.; Booth, N.A. Evidence for an active fibrinolytic system in normal human bone marrow. *Br. J. Haematol.* **1996**, *93*, 170–176. [[CrossRef](#)]
26. Chitteti, B.R.; Kacena, M.A.; Voytik-Harbin, S.L.; Srouf, E.F. Modulation of hematopoietic progenitor cell fate in vitro by varying collagen oligomer matrix stiffness in the presence or absence of osteoblasts. *J. Immunol. Methods* **2015**, *425*, 108–113. [[CrossRef](#)]
27. Witkowska-Zimny, M.; Walenko, K.; Wrobel, E.; Mrowka, P.; Mikulska, A.; Przybylski, J. Effect of substrate stiffness on the osteogenic differentiation of bone marrow stem cells and bone-derived cells. *Cell Biol. Int.* **2013**, *37*, 608–616. [[CrossRef](#)]

28. Aguilar, A.; Pertuy, F.; Eckly, A.; Strassel, C.; Collin, D.; Gachet, C.; Lanza, F.; Leon, C. Importance of environmental stiffness for megakaryocyte differentiation and proplatelet formation. *Blood* **2016**, *128*, 2022–2032. [[CrossRef](#)]
29. Shin, J.W.; Swift, J.; Spinler, K.R.; Discher, D.E. Myosin-II inhibition and soft 2D matrix maximize multinucleation and cellular projections typical of platelet-producing megakaryocytes. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 11458–11463. [[CrossRef](#)]
30. Abbonante, V.; Di Buduo, C.A.; Gruppi, C.; De Maria, C.; Spedden, E.; De Acutis, A.; Staii, C.; Raspanti, M.; Vozzi, G.; Kaplan, D.L.; et al. A new path to platelet production through matrix sensing. *Haematologica* **2017**, *102*, 1150–1160. [[CrossRef](#)]
31. Di Buduo, C.A.; Wray, L.S.; Tozzi, L.; Malara, A.; Chen, Y.; Ghezzi, C.E.; Smoot, D.; Sfara, C.; Antonelli, A.; Spedden, E.; et al. Programmable 3D silk bone marrow niche for platelet generation ex vivo and modeling of megakaryopoiesis pathologies. *Blood* **2015**, *125*, 2254–2264. [[CrossRef](#)] [[PubMed](#)]
32. Hynes, R.O. Integrins: Bidirectional, allosteric signaling machines. *Cell* **2002**, *110*, 673–687. [[CrossRef](#)]
33. Fässler, R.; Meyer, M. Consequences of lack of beta 1 integrin gene expression in mice. *Genes Dev.* **1995**, *9*, 1896–1908. [[CrossRef](#)] [[PubMed](#)]
34. Chen, J.; Diacovo, T.G.; Grenache, D.G.; Santoro, S.A.; Zutter, M.M. The alpha(2) integrin subunit-deficient mouse: A multifaceted phenotype including defects of branching morphogenesis and hemostasis. *Am. J. Pathol.* **2002**, *161*, 337–344. [[CrossRef](#)]
35. Arroyo, A.G.; Yang, J.T.; Rayburn, H.; Hynes, R.O. Alpha4 integrins regulate the proliferation/differentiation balance of multilineage hematopoietic progenitors in vivo. *Immunity* **1999**, *11*, 555–566. [[CrossRef](#)]
36. Arroyo, A.G.; Yang, J.T.; Rayburn, H.; Hynes, R.O. Differential requirements for alpha4 integrins during fetal and adult hematopoiesis. *Cell* **1996**, *85*, 997–1008. [[CrossRef](#)]
37. Winograd-Katz, S.E.; Fässler, R.; Geiger, B.; Legate, K.R. The integrin adhesome: From genes and proteins to human disease. *Nat. Rev. Mol. Cell Biol.* **2014**, *15*, 273–288. [[CrossRef](#)]
38. Eshghi, S.; Vogelesang, M.G.; Hynes, R.O.; Griffith, L.G.; Lodish, H.F. Alpha4beta1 integrin and erythropoietin mediate temporally distinct steps in erythropoiesis: Integrins in red cell development. *J. Cell Biol.* **2007**, *177*, 871–880. [[CrossRef](#)]
39. Vuillet-Gaugler, M.H.; Breton-Gorius, J.; Vainchenker, W.; Guichard, J.; Leroy, C.; Tchernia, G.; Coulombel, L. Loss of attachment to fibronectin with terminal human erythroid differentiation. *Blood* **1990**, *75*, 865–873. [[CrossRef](#)]
40. Roseblatt, M.; Vuillet-Gaugler, M.H.; Leroy, C.; Coulombel, L. Coexpression of two fibronectin receptors, VLA-4 and VLA-5, by immature human erythroblastic precursor cells. *J. Clin. Invest.* **1991**, *87*, 6–11. [[CrossRef](#)]
41. Hu, J.; Liu, J.; Xue, F.; Halverson, G.; Reid, M.; Guo, A.; Chen, L.; Raza, A.; Galili, N.; Jaffray, J.; et al. Isolation and functional characterization of human erythroblasts at distinct stages: Implications for understanding of normal and disordered erythropoiesis in vivo. *Blood* **2013**, *121*, 3246–3253. [[CrossRef](#)] [[PubMed](#)]
42. Yanai, N.; Sekine, C.; Yagita, H.; Obinata, M. Roles for integrin very late activation antigen-4 in stroma-dependent erythropoiesis. *Blood* **1994**, *83*, 2844–2850. [[CrossRef](#)] [[PubMed](#)]
43. Ulyanova, T.; Georgolopoulos, G.; Papayannopoulou, T. Reappraising the role of α_5 integrin and the microenvironmental support in stress erythropoiesis. *Exp. Hematol.* **2019**. [[CrossRef](#)] [[PubMed](#)]
44. Ulyanova, T.; Padilla, S.M.; Papayannopoulou, T. Stage-specific functional roles of integrins in murine erythropoiesis. *Exp. Hematol.* **2014**, *42*, 404–409.e404. [[CrossRef](#)]
45. Ulyanova, T.; Jiang, Y.; Padilla, S.; Nakamoto, B.; Papayannopoulou, T. Combinatorial and distinct roles of α_5 and α_4 integrins in stress erythropoiesis in mice. *Blood* **2011**, *117*, 975–985. [[CrossRef](#)]
46. Pallotta, I.; Lovett, M.; Rice, W.; Kaplan, D.L.; Balduini, A. Bone marrow osteoblastic niche: A new model to study physiological regulation of megakaryopoiesis. *PLoS ONE* **2009**, *4*, e8359. [[CrossRef](#)]
47. Sabri, S.; Jandrot-Perrus, M.; Bertoglio, J.; Farndale, R.W.; Mas, V.M.; Debili, N.; Vainchenker, W. Differential regulation of actin stress fiber assembly and proplatelet formation by alpha2beta1 integrin and GPVI in human megakaryocytes. *Blood* **2004**, *104*, 3117–3125. [[CrossRef](#)]
48. Steevels, T.A.; Westerlaken, G.H.; Tijssen, M.R.; Coffey, P.J.; Lenting, P.J.; Akkerman, J.W.; Meyaard, L. Co-expression of the collagen receptors leukocyte-associated immunoglobulin-like receptor-1 and glycoprotein VI on a subset of megakaryoblasts. *Haematologica* **2010**, *95*, 2005–2012. [[CrossRef](#)]

49. Abbonante, V.; Gruppi, C.; Rubel, D.; Gross, O.; Moratti, R.; Balduini, A. Discoidin domain receptor 1 protein is a novel modulator of megakaryocyte-collagen interactions. *J. Biol. Chem.* **2013**, *288*, 16738–16746. [[CrossRef](#)]
50. Molla, A.; Mossuz, P.; Berthier, R. Extracellular matrix receptors and the differentiation of human megakaryocytes in vitro. *Leuk Lymphoma* **1999**, *33*, 15–23. [[CrossRef](#)]
51. Semeniak, D.; Kulawig, R.; Stegner, D.; Meyer, I.; Schwiebert, S.; Bosing, H.; Eckes, B.; Nieswandt, B.; Schulze, H. Proplatelet formation is selectively inhibited by collagen type I through Syk-independent GPVI signaling. *J. Cell Sci.* **2016**, *129*, 3473–3484. [[CrossRef](#)] [[PubMed](#)]
52. Balduini, A.; Pallotta, I.; Malara, A.; Lova, P.; Pecci, A.; Viarengo, G.; Balduini, C.L.; Torti, M. Adhesive receptors, extracellular proteins and myosin IIA orchestrate proplatelet formation by human megakaryocytes. *J. Thromb. Haemost* **2008**, *6*, 1900–1907. [[CrossRef](#)] [[PubMed](#)]
53. Chang, Y.; Aurade, F.; Larbret, F.; Zhang, Y.; Le Couedic, J.P.; Momeux, L.; Larghero, J.; Bertoglio, J.; Louache, F.; Cramer, E.; et al. Proplatelet formation is regulated by the Rho/ROCK pathway. *Blood* **2007**, *109*, 4229–4236. [[CrossRef](#)] [[PubMed](#)]
54. Malara, A.; Gruppi, C.; Pallotta, I.; Spedden, E.; Tenni, R.; Raspanti, M.; Kaplan, D.; Tira, M.E.; Staii, C.; Balduini, A. Extracellular matrix structure and nano-mechanics determine megakaryocyte function. *Blood* **2011**, *118*, 4449–4453. [[CrossRef](#)] [[PubMed](#)]
55. Lucero, H.A.; Kagan, H.M. Lysyl oxidase: An oxidative enzyme and effector of cell function. *Cell Mol. Life Sci.* **2006**, *63*, 2304–2316. [[CrossRef](#)]
56. Eliades, A.; Papadantonakis, N.; Bhupatiraju, A.; BurrIDGE, K.A.; Johnston-Cox, H.A.; Migliaccio, A.R.; Crispino, J.D.; Lucero, H.A.; Trackman, P.C.; Ravid, K. Control of megakaryocyte expansion and bone marrow fibrosis by lysyl oxidase. *J. Biol. Chem.* **2011**, *286*, 27630–27638. [[CrossRef](#)]
57. Leiva, O.; Ng, S.K.; Matsuura, S.; Chitalia, V.; Lucero, H.; Findlay, A.; Turner, C.; Jarolimek, W.; Ravid, K. Novel lysyl oxidase inhibitors attenuate hallmarks of primary myelofibrosis in mice. *Int. J. Hematol.* **2019**, *110*, 699–708. [[CrossRef](#)]
58. Jiang, F.; Jia, Y.; Cohen, I. Fibronectin- and protein kinase C-mediated activation of ERK/MAPK are essential for proplateletlike formation. *Blood* **2002**, *99*, 3579–3584. [[CrossRef](#)]
59. Han, P.; Guo, X.H.; Story, C.J. Enhanced expansion and maturation of megakaryocytic progenitors by fibronectin. *Cytotherapy* **2002**, *4*, 277–283. [[CrossRef](#)]
60. Berthier, R.; Jacquier-Sarlin, M.; Schweitzer, A.; Block, M.R.; Molla, A. Adhesion of mature polyploid megakaryocytes to fibronectin is mediated by beta 1 integrins and leads to cell damage. *Exp. Cell Res.* **1998**, *242*, 315–327. [[CrossRef](#)]
61. Fox, N.E.; Kaushansky, K. Engagement of integrin alpha4beta1 enhances thrombopoietin-induced megakaryopoiesis. *Exp. Hematol.* **2005**, *33*, 94–99. [[CrossRef](#)] [[PubMed](#)]
62. Mitra, S.K.; Hanson, D.A.; Schlaepfer, D.D. Focal adhesion kinase: In command and control of cell motility. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 56–68. [[CrossRef](#)] [[PubMed](#)]
63. Chen, Z.; Naveiras, O.; Balduini, A.; Mammoto, A.; Conti, M.A.; Adelstein, R.S.; Ingber, D.; Daley, G.Q.; Shivdasani, R.A. The May-Hegglin anomaly gene MYH9 is a negative regulator of platelet biogenesis modulated by the Rho-ROCK pathway. *Blood* **2007**, *110*, 171–179. [[CrossRef](#)] [[PubMed](#)]
64. Ranade, S.S.; Syeda, R.; Patapoutian, A. Mechanically Activated Ion Channels. *Neuron* **2015**, *87*, 1162–1179. [[CrossRef](#)] [[PubMed](#)]
65. Trebak, M.; Vazquez, G.; Bird, G.S.; Putney, J.W., Jr. The TRPC3/6/7 subfamily of cation channels. *Cell Calcium.* **2003**, *33*, 451–461. [[CrossRef](#)]
66. Zhu, X.; Jiang, M.; Peyton, M.; Boulay, G.; Hurst, R.; Stefani, E.; Birnbaumer, L. trp, a novel mammalian gene family essential for agonist-activated capacitative Ca²⁺ entry. *Cell* **1996**, *85*, 661–671. [[CrossRef](#)]
67. Liedtke, W.; Choe, Y.; Marti-Renom, M.A.; Bell, A.M.; Denis, C.S.; Sali, A.; Hudspeth, A.J.; Friedman, J.M.; Heller, S. Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. *Cell* **2000**, *103*, 525–535. [[CrossRef](#)]
68. Vriens, J.; Watanabe, H.; Janssens, A.; Droogmans, G.; Voets, T.; Nilius, B. Cell swelling, heat, and chemical agonists use distinct pathways for the activation of the cation channel TRPV4. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 396–401. [[CrossRef](#)]
69. Strotmann, R.; Harteneck, C.; Nunnenmacher, K.; Schultz, G.; Plant, T.D. OTRPC4, a nonselective cation channel that confers sensitivity to extracellular osmolarity. *Nat. Cell Biol.* **2000**, *2*, 695–702. [[CrossRef](#)]

70. Wegierski, T.; Lewandrowski, U.; Muller, B.; Sickmann, A.; Walz, G. Tyrosine phosphorylation modulates the activity of TRPV4 in response to defined stimuli. *J. Biol. Chem.* **2009**, *284*, 2923–2933. [[CrossRef](#)]
71. Grace, M.S.; Lieu, T.; Darby, B.; Abogadie, F.C.; Veldhuis, N.; Bunnett, N.W.; McIntyre, P. The tyrosine kinase inhibitor bafetinib inhibits PAR2-induced activation of TRPV4 channels in vitro and pain in vivo. *Br. J. Pharm.* **2014**, *171*, 3881–3894. [[CrossRef](#)]
72. Poole, D.P.; Amadesi, S.; Veldhuis, N.A.; Abogadie, F.C.; Lieu, T.; Darby, W.; Liedtke, W.; Lew, M.J.; McIntyre, P.; Bunnett, N.W. Protease-activated receptor 2 (PAR2) protein and transient receptor potential vanilloid 4 (TRPV4) protein coupling is required for sustained inflammatory signaling. *J. Biol. Chem.* **2013**, *288*, 5790–5802. [[CrossRef](#)]
73. Coste, B.; Mathur, J.; Schmidt, M.; Earley, T.J.; Ranade, S.; Petrus, M.J.; Dubin, A.E.; Patapoutian, A. Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. *Science* **2010**, *330*, 55–60. [[CrossRef](#)] [[PubMed](#)]
74. Hirschler-Laszkiwicz, I.; Tong, Q.; Waybill, K.; Conrad, K.; Keefer, K.; Zhang, W.; Chen, S.-j.; Cheung, J.Y.; Miller, B.A. The transient receptor potential (TRP) channel TRPC3 TRP domain and AMP-activated protein kinase binding site are required for TRPC3 activation by erythropoietin. *J. Biol. Chem.* **2011**, *286*, 30636–30646. [[CrossRef](#)] [[PubMed](#)]
75. Foller, M.; Kasinathan, R.S.; Koka, S.; Lang, C.; Shumilina, E.; Birnbaumer, L.; Lang, F.; Huber, S.M. TRPC6 contributes to the Ca(2+) leak of human erythrocytes. *Cell. Physiol. Biochem. : Int. J. Exp. Cell. Physiol. Biochem. Pharmacol.* **2008**, *21*, 183–192. [[CrossRef](#)] [[PubMed](#)]
76. Chu, X.; Tong, Q.; Cheung, J.Y.; Wozney, J.; Conrad, K.; Mazack, V.; Zhang, W.; Stahl, R.; Barber, D.L.; Miller, B.A. Interaction of TRPC2 and TRPC6 in erythropoietin modulation of calcium influx. *J. Biol. Chem.* **2004**, *279*, 10514–10522. [[CrossRef](#)]
77. Hirschler-Laszkiwicz, I.; Tong, Q.; Conrad, K.; Zhang, W.; Flint, W.W.; Barber, A.J.; Barber, D.L.; Cheung, J.Y.; Miller, B.A. TRPC3 activation by erythropoietin is modulated by TRPC6. *J. Biol. Chem.* **2009**, *284*, 4567–4581. [[CrossRef](#)] [[PubMed](#)]
78. Clark, K.; Middelbeek, J.; van Leeuwen, F.N. Interplay between TRP channels and the cytoskeleton in health and disease. *Eur. J. Cell Biol.* **2008**, *87*, 631–640. [[CrossRef](#)]
79. Goswami, C.; Hucho, T. Submembrane microtubule cytoskeleton: Biochemical and functional interplay of TRP channels with the cytoskeleton. *FEBS J.* **2008**, *275*, 4684–4699. [[CrossRef](#)]
80. Nikolaev, Y.A.; Cox, C.D.; Ridone, P.; Rohde, P.R.; Cordero-Morales, J.F.; Vásquez, V.; Laver, D.R.; Martinac, B. Mammalian TRP ion channels are insensitive to membrane stretch. *J. Cell Sci.* **2019**, *132*, jcs238360. [[CrossRef](#)]
81. Zarychanski, R.; Schulz, V.P.; Houston, B.L.; Maksimova, Y.; Houston, D.S.; Smith, B.; Rinehart, J.; Gallagher, P.G. Mutations in the mechanotransduction protein PIEZO1 are associated with hereditary xerocytosis. *Blood* **2012**, *120*, 1908–1915. [[CrossRef](#)] [[PubMed](#)]
82. Andolfo, I.; Alper, S.L.; De Franceschi, L.; Auriemma, C.; Russo, R.; De Falco, L.; Vallefucio, F.; Esposito, M.R.; Vandorpe, D.H.; Shmukler, B.E.; et al. Multiple clinical forms of dehydrated hereditary stomatocytosis arise from mutations in PIEZO1. *Blood* **2013**, *121*, 3925–3935. [[CrossRef](#)] [[PubMed](#)]
83. Murthy, S.E.; Dubin, A.E.; Patapoutian, A. Piezos thrive under pressure: Mechanically activated ion channels in health and disease. *Nat. Rev. Mol. Cell Biol.* **2017**, *18*, 771–783. [[CrossRef](#)] [[PubMed](#)]
84. Lewis, A.H.; Grandl, J. Mechanical sensitivity of Piezo1 ion channels can be tuned by cellular membrane tension. *Elife* **2015**, *4*. [[CrossRef](#)] [[PubMed](#)]
85. Kuchel, P.W.; Shishmarev, D. Accelerating metabolism and transmembrane cation flux by distorting red blood cells. *Sci. Adv.* **2017**, *3*, eaao1016. [[CrossRef](#)] [[PubMed](#)]
86. Svetina, S.; Svelc Kebe, T.; Bozic, B. A Model of Piezo1-Based Regulation of Red Blood Cell Volume. *Biophys. J.* **2019**, *116*, 151–164. [[CrossRef](#)]
87. Bae, C.; Sachs, F.; Gottlieb, P.A. The mechanosensitive ion channel Piezo1 is inhibited by the peptide GsMTx4. *Biochemistry* **2011**, *50*, 6295–6300. [[CrossRef](#)]
88. Syeda, R.; Xu, J.; Dubin, A.E.; Coste, B.; Mathur, J.; Huynh, T.; Matzen, J.; Lao, J.; Tully, D.C.; Engels, I.H.; et al. Chemical activation of the mechanotransduction channel Piezo1. *Elife* **2015**, *4*. [[CrossRef](#)]
89. Danielczok, J.G.; Terriac, E.; Hertz, L.; Petkova-Kirova, P.; Lautenschlager, F.; Laschke, M.W.; Kaestner, L. Red Blood Cell Passage of Small Capillaries Is Associated with Transient Ca(2+)-mediated Adaptations. *Front. Physiol.* **2017**, *8*, 979. [[CrossRef](#)]

90. Cahalan, S.M.; Lukacs, V.; Ranade, S.S.; Chien, S.; Bandell, M.; Patapoutian, A. Piezo1 links mechanical forces to red blood cell volume. *Elife* **2015**, *4*. [[CrossRef](#)]
91. Grgic, I.; Kaistha, B.P.; Paschen, S.; Kaistha, A.; Busch, C.; Si, H.; Köhler, K.; Elsässer, H.-P.; Hoyer, J.; Köhler, R. Disruption of the Gardos channel (KCa3.1) in mice causes subtle erythrocyte macrocytosis and progressive splenomegaly. *Pflug. Arch. : Eur. J. Physiol.* **2009**, *458*, 291–302. [[CrossRef](#)] [[PubMed](#)]
92. Fermo, E.; Bogdanova, A.; Petkova-Kirova, P.; Zaninoni, A.; Marcello, A.P.; Makhro, A.; Hänggi, P.; Hertz, L.; Danielczok, J.; Vercellati, C.; et al. ‘Gardos Channelopathy’: A variant of hereditary Stomatocytosis with complex molecular regulation. *Sci. Rep.* **2017**, *7*, 1744. [[CrossRef](#)] [[PubMed](#)]
93. Andolfo, I.; Russo, R.; Manna, F.; Shmukler, B.E.; Gambale, A.; Vitiello, G.; De Rosa, G.; Brugnara, C.; Alper, S.L.; Snyder, L.M.; et al. Novel Gardos channel mutations linked to dehydrated hereditary stomatocytosis (xerocytosis). *Am. J. Hematol.* **2015**, *90*, 921–926. [[CrossRef](#)]
94. Caulier, A.; Jankovsky, N.; Demont, Y.; Ouled-Haddou, H.; Demagny, J.; Guitton, C.; Merlusca, L.; Lebon, D.; Vong, P.; Aubry, A.; et al. PIEZO1 activation delays erythroid differentiation of normal and Hereditary Xerocytosis-derived human progenitors. *Haematologica* **2019**. [[CrossRef](#)] [[PubMed](#)]
95. Ramanathan, G.; Mannhalter, C. Increased expression of transient receptor potential canonical 6 (TRPC6) in differentiating human megakaryocytes. *Cell Biol. Int.* **2016**, *40*, 223–231. [[CrossRef](#)] [[PubMed](#)]
96. Ilkan, Z.; Wright, J.R.; Goodall, A.H.; Gibbins, J.M.; Jones, C.I.; Mahaut-Smith, M.P. Evidence for shear-mediated Ca(2+) entry through mechanosensitive cation channels in human platelets and a megakaryocytic cell line. *J. Biol. Chem.* **2017**, *292*, 9204–9217. [[CrossRef](#)] [[PubMed](#)]
97. Jardin, I.; Gomez, L.J.; Salido, G.M.; Rosado, J.A. Dynamic interaction of hTRPC6 with the Orai1-STIM1 complex or hTRPC3 mediates its role in capacitative or non-capacitative Ca(2+) entry pathways. *Biochem. J.* **2009**, *420*, 267–276. [[CrossRef](#)] [[PubMed](#)]
98. Harper, M.T.; Sage, S.O. Src family tyrosine kinases activate thrombin-induced non-capacitative cation entry in human platelets. *Platelets* **2010**, *21*, 445–450. [[CrossRef](#)]
99. Dionisio, N.; Albarran, L.; Berna-Erro, A.; Hernandez-Cruz, J.M.; Salido, G.M.; Rosado, J.A. Functional role of the calmodulin- and inositol 1,4,5-trisphosphate receptor-binding (CIRB) site of TRPC6 in human platelet activation. *Cell Signal.* **2011**, *23*, 1850–1856. [[CrossRef](#)]
100. Ramanathan, G.; Gupta, S.; Thielmann, I.; Pleines, I.; Varga-Szabo, D.; May, F.; Mannhalter, C.; Dietrich, A.; Nieswandt, B.; Braun, A. Defective diacylglycerol-induced Ca²⁺ entry but normal agonist-induced activation responses in TRPC6-deficient mouse platelets. *J. Thromb. Haemost.* **2012**, *10*, 419–429. [[CrossRef](#)]



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).