Pathophysiological Significance of Store-Operated Calcium Entry in Megakaryocyte Function: Opening New Paths for Understanding the Role of Calcium in Thrombopoiesis

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Abstract: Store-Operated Calcium Entry (SOCE) is a universal calcium (Ca^{2+}) influx mechanism expressed by several different cell types. It is now known that Stromal Interaction Molecule (STIM), the Ca^{2+} sensor of the intracellular compartments, together with Orai and Transient Receptor Potential Canonical (TRPC), the subunits of Ca^{2+} permeable channels on the plasma membrane, cooperate in regulating multiple cellular functions as diverse as proliferation, differentiation, migration, gene expression, and many others, depending on the cell type. In particular, a growing body of evidences suggests that a tight control of SOCE expression and function is achieved by megakaryocytes along their route from hematopoietic stem cells to platelet production. This review attempts to provide an overview about the SOCE dynamics in megakaryocyte development, with a focus on most recent findings related to its involvement in physiological and pathological thrombopoiesis.

Keywords: megakaryocyte; platelet production; calcium; SOCE; calreticulin; myelofibrosis

1. Introduction

The calcium ion (Ca^{2+}) is an ubiquitous signaling entity which plays key role in regulating the functions of virtually all cell types, including proliferation, differentiation, exocytosis, gene transcription, migration and apoptosis [1,2]. The intracellular responses are regulated by a tight control of intracellular cytoplasmic Ca^{2+} concentration ([Ca^{2+}]) by the finely tuned interplay of several Ca^{2+}-transporting proteins, such as Ca^{2+} pumps, Ca^{2+} binding proteins and Ca^{2+} permeable channels. In resting states, the sarco/endoplasmic reticulum (SR/ER) Ca^{2+}-ATPase (SERCA) pump, along with plasma membrane Ca^{2+}-ATPase (PMCA) and the Na^{+}/Ca^{2+} exchanger (NCX), determine the basal [Ca^{2+}]; while, upon stimulation by extracellular signals, [Ca^{2+}] increases by Ca^{2+} release from intracellular stores or extracellular Ca^{2+} influx into the cell [3].

The main intracellular Ca^{2+} store is represented by the SR/ER. However, using genetically targeted Ca^{2+} reporter proteins, like aequorin and the cameleons, together with detailed immunocytochemical mapping and functional assays, contributions from additional organelles such as the Golgi apparatus, the lysosomes, the mitochondria, the nuclear envelope, and the secretory granules have been identified [1,4-6].
Ca\(^{2+}\) release from intracellular stores is usually determined by the activation of the phospholipase C (PLC) pathway by G-Protein Coupled Receptors (GPCRs) or Tyrosine Kinase Receptors (TKRs) [7]. PLC cleaves phosphatidylinositol 4,5-bisphosphate (PIP2) into the second messengers inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). DAG engages both protein kinase C and non-selective cation channels on the cellular membrane, such as Transient Receptor Potential Canonical (TRPC) 3, TRPC6 and TRPC7, whereas IP3 rapidly diffuses within the cytosol to bind to and open ER-embedded IP3 receptors (IP3Rs). IP3Rs, in turn, serve as Ca\(^{2+}\)-permeable channels to release luminal stored Ca\(^{2+}\). In addition to IP3, cyclic adenosine diphosphate (ADP) ribose triggers Ca\(^{2+}\) release from the ER by gating ryanodine receptors (RyRs) [8], while nicotinic acid adenine dinucleotide phosphate mobilizes Ca\(^{2+}\) stored within the acidic Ca\(^{2+}\) stores of the endolysosomal system [9].

Extracellular Ca\(^{2+}\) influx can occur through various pathways. A variety of different Ca\(^{2+}\)-permeable channels have been found to coexist in the plasma membrane including voltage-operated channels (VOCs), second messenger-operated channels (SMOCs), receptor-operated channels (ROCs) and store-operated channels (SOCs) [3]. VOCs are activated by membrane depolarization and are found in excitable cells, like nerve and muscle cells, but are largely excluded from nonexcitable cells. SMOCs, found in some excitable and nonexcitable cells, are activated by small messenger molecules, the most common being IP3, cyclic nucleotides, and lipid-derived messengers (DAG, arachidonic acid and its metabolites). ROCs, preponderant in excitable cells, open rapidly upon binding an external ligand that is usually a neurotransmitter or a hormone. Finally, SOCs are activated by the depletion of intracellular Ca\(^{2+}\) stores, according to a mechanism termed Store-Operated Ca\(^{2+}\) Entry (SOCE). SOCs appear to be widespread in nonexcitable cells, although they are also present in excitable cells [10–12], existing in all eukaryotes from yeast [13] to humans [14], thus probably representing the primordial and best preserved Ca\(^{2+}\) entry pathway.

Although originally identified as a mechanism for ensuring the refilling of intracellular stores following Ca\(^{2+}\) release [15,16], SOCE is now directly linked to the activation of specific cellular functions, differently regulated depending on the stimulus and the cell type [10], including immune system activation [17], fluid secretion in salivary gland acinar cells [18], neurogenesis and neuronal excitability [12,19], cancer cell migration and metastasis [20], endothelial cell proliferation [21], skeletal muscle contractility [22], smooth muscle migration and proliferation [23,24], cardiac hypertrophy [25], cell cycle and cell proliferation [26], and gene expression [27]. Further, increasing evidences have recently identified the contribution of SOCE in thrombopoiesis, the process that ensures the differentiation of megakaryocytes, the platelet precursors, and final platelet production [28].

Here we will review the mechanisms of SOCE and discuss the most recent findings regarding its involvement in regulating different megakaryocyte functions, from proliferation to platelet formation. Finally, we will provide evidence that an alteration of SOCE activity may concur to the development of human pathologies.

2. Dissecting the Molecular Mechanisms of Store-Operated Calcium Entry

The physiological hallmark of SOCE is the long-lasting plateau phase that follows the initial IP3-dependent intracellular Ca\(^{2+}\) release induced by extracellular stimulation. Specifically, the first signal is generated upon binding of cytokines, growth factors, hormones, and neurotransmitters to their specific receptors, which leads to the generation of the second messenger IP3 and consequent mobilization of the IP3-sensitive Ca\(^{2+}\) pool. In the second phase, the decrease in ER Ca\(^{2+}\) content causes the activation of plasma membrane Ca\(^{2+}\) channels resulting in the influx of extracellular Ca\(^{2+}\) inside the cells [10,29]. This mechanism was first described by Putney in 1986 as “capacitative Ca\(^{2+}\) entry”, when he proposed that the amount of Ca\(^{2+}\) in the stores of acinar parathyroid cells controls the extent of Ca\(^{2+}\) influx and store refilling and compared it to the arrangement of resistor (channel) and capacitor (Ca\(^{2+}\) store) in an electrical circuitry [15]. Nearly after, the evidence of the existence of a store-operated Ca\(^{2+}\)-selective membrane current with a largely positive reversal potential \(E_{rev} = +60/+70\) mV, that arose in response to different Ca\(^{2+}\) store depletion strategies, further supported the hypothesis that
highly selective Ca\textsuperscript{2+}-Release Activated Ca\textsuperscript{2+} (CRAC) channels could be activated in response to ER Ca\textsuperscript{2+} emptying \cite{30,31}. The molecular structure of CRAC channels has been fully dissected in the last decade by carrying out an extensive function-based genetic screen by systematic Ribonucleic Acid (RNA) interference conducted on a subset of candidate genes in \textit{Drosophila} S2 cells and HeLa cells \cite{32–35}. This approach led to the identification and characterization of the Stromal Interaction Molecule (STIM) family, which represents the ER Ca\textsuperscript{2+} sensor, and of the Orai family, which provides the pore-forming subunit of CRAC channels, that cooperate in an elegant signaling mechanism to ensure SOCE activation.

2.1. Stromal Interaction Molecules: The Calcium Sensor of Intracellular Stores

STIMs, first identified in 2005, function as Ca\textsuperscript{2+} sensors in the ER and control CRAC channel opening in both quiescent and stimulated cells \cite{32,33,36,37}. This family encompasses two members, STIM1 and STIM2; however, most studies have concluded that the ER-localized STIM1 is the main isoform involved in SOCE activation upon extracellular stimulation \cite{32,38,39}, while STIM2 controls Ca\textsuperscript{2+} entry in resting cells \cite{40}.

STIM1 is a single-pass transmembrane (TM) protein of 665 amino acids and \( \approx 77 \) kDa that is abundant in the ER membrane \cite{32,39}, but can be also expressed on the plasma membrane, although at a minor extent \cite{36,41}. When embedded within the ER membrane, STIM1 is oriented such that the N-terminus appears within the lumen and the C-terminus in the cytoplasm. The protein is comprised of several identifiable structural and functional motifs that are shared with STIM2 \cite{10}. The luminal side contains a Ca\textsuperscript{2+} binding canonical EF-hand domain (cEF), which confers the protein with sensitivity to ER Ca\textsuperscript{2+} levels, a hidden non-canonical EF-hand domain (hEF) that does not bind Ca\textsuperscript{2+}, and a sterile-\( \alpha \) motif (SAM), that is required for protein-protein interaction during the oligomerization process (see below) \cite{10,32,33}. SAM is followed by a TM domain which is followed by three conserved CC domains (CC1, CC2 and CC3) and polybasic lysine-rich (K) domain at the very end of the C-terminus which mediate, respectively, STIM1 binding to Orai1 and membrane phospholipids. More specifically, Orai1 is recruited and gated by the CRAC activation domain (CAD; also termed STIM-Orai-activating region or SOAR or coiled-coil domain b9 or CCb9) of STIM1, which encompasses CC2 and CC3, while the K-domain anchors STIM1 to the inner leaflet of the plasma membrane \cite{10,32,42,43}. STIM2, the second member of the vertebrate STIM protein family, is exclusively present in the ER membrane \cite{44}, shows 61% amino acid homology and similar domain architecture to STIM1 \cite{45}, but presents a lower Ca\textsuperscript{2+} binding affinity \cite{46}. The variety of STIM proteins is further enhanced by the existence of diverse splice variants, namely STIM1L \cite{47} and three STIM2 splice variants, STIM2.1 (also known as STIM2\( \beta \)), STIM2.2 (or STIM2\( \alpha \)) and STIM2.3 \cite{48,49}. Of these, STIM2.1 is a positive regulator of Orai1, while STIM2.2 inhibits CRAC currents and the function of STIM2.3 is still unknown.

2.2. The Interplay between Stromal Interaction Molecule and Orai Activates the Complex Choreography of Store-Operated Calcium Entry

In mammals there are three Orai genes that encode for Orai1, 2 and 3 proteins which function as pore forming subunits of CRAC channels in different cellular contexts \cite{10,12,20,42,50}. The name Orai was given on the basis of Greek mythology (Orai are the keepers of heaven’s gate) after they were established as the long sought mediator of CRAC currents in immune cells \cite{51}. Each Orai channel consists in a \( \approx 30 \) kDa monomer that comprises four TM domains flanked by cytosolic N- and C-termini and linked by one intracellular and two extracellular loops. The TM segments of each Orai isoform share \( 81\%–87\% \) pairwise sequence identity \cite{52}. However, only Orai1 has an N-terminal proline- and arginine-rich region that could take part to channel gating \cite{52}. The C-terminus of each Orai isoform contains a CC domain which is also required for the physical interaction with STIM1 \cite{53,54}. Although it has long been thought that Orai subunits are assembled into a tetrameric channel \cite{10}, the crystal structure of \textit{Drosophila} Orai (dOrai) unveiled a hexameric organization \cite{55}. The six subunits are arranged around a central pore, which is lined exclusively by TM1 and by an
which represents the archetypal store-operated current, has been defined as follows [10,29,60]: Lack of
which coordinate anions, a rather unusual feature for a cation-selective channel [55]. The channel
(TM4 constitutes the outermost segment, the one which is presumably most exposed to membrane
pore is flanked by three concentric rings subsequently contributed by TM2 to TM4, whereby TM2
process [56]. A ring of glutamate residues (E106) at the outer mouth of the channel pore constitutes
voltage-dependent activation, prominent inward rectification at negative potentials, reversal potential
\( \alpha \) from 1 to 43 pS [29]. The I_{CRAC} to Ca_{2+} exhibited slightly biophysical properties as respect to the I_{SOC}.

The molecular architecture of dOrai needs to be confirmed in humans, but it provides a
solid framework to decipher the complex interaction between STIM and Orai proteins.

When Ca_{2+} stores are full and the ER Ca_{2+} concentration ranges between 400 and 600 \( \mu \)M, STIM1 forms dimers which are homogeneously distributed throughout the ER membrane due to their ability to rapidly diffuse along the microtubules [10]. Upon IP3-dependent Ca_{2+} mobilization, Ca_{2+} dissociates from the cEF hand, thereby triggering a complex sequence of intra- and intermolecular interactions that culminate in STIM1 activation, multimerization, and relocation into defined ER-plasma membrane junctions. Herein, STIM1 may finally bind to and gate Orai1, thereby activating the Calcium Release-Activated Calcium Current (I_{CRAC}) [10,42,59]. Each Orai isoform carries a Ca_{2+}-selective current with well-defined biophysical and pharmacological features. The fingerprint of the I_{CRAC}, which represents the archetypal store-operated current, has been defined as follows [10,29,60]: Lack of voltage-dependent activation, prominent inward rectification at negative potentials, reversal potential (\( E_{rev} \)) > +60 mV, \( \text{Ca}_{2+}^{2+}:\text{Na}^+ \) permeability ratio of 1000:1, unitary Ca_{2+} conductance of 10–35 F, fast Ca_{2+}-dependent inactivation (CDI), high sensitivity to trivalent cation block and biphasic sensitivity to 2-aminoethoxydiphenyl borate (2-APB; activates at <10 \( \mu \)M, but inhibits at 50–100 \( \mu \)M). Orai1 mediates the I_{CRAC} in a growing number of cell types, including, but not limited to, immune cells [61], endothelial cells [62], vascular smooth muscle cells [63], melanocytes [64], microglia [65] and hepatocytes [66].

Although very similar in structure, STIM1 and STIM2 differ in their sensitivity to ER Ca_{2+} levels, their Ca_{2+} dissociation constants (\( K_d \)) being, respectively, 200 and 400 \( \mu \)M [40]. As the ER Ca_{2+} concentration ([Ca_{2+}]_ER) ranges between 400 and 600 \( \mu \)M, STIM2 is activated by significantly smaller depletion of the luminal Ca_{2+} pool as compared to STIM1 [40]; moreover, the EF-SAM domains of STIM2 undergoes slower unfolding and self-association kinetics upon Ca_{2+} withdrawal, which reflects in slower and less efficient Orai1 activation [46,67]. It turns out that, while STIM1 drives SOCE upon massive emptying of the ER Ca_{2+} pool, STIM2 controls basal Ca_{2+} entry and sustains the physiological Ca_{2+} oscillations arising in response to moderate-to-weak stimulation [40,68]. The stoichiometry of STIM1-Orai1 coupling is still matter of debate as the I_{CRAC} is exquisitely sensitive to the number of STIM1 subunits interacting with the Orai1 hexamer [69,70]. Several studies reached the conclusion that maximal I_{CRAC} activation occurs at a ratio of 2:1 of STIM1 and Orai1, respectively, while others argued in favor of a 1:1 stoiochiometric ratio [69,71].

3. Transient Receptor Potential Canonical Channels: Additional Components of Store-Operated Calcium Entry

The I_{CRAC} is not the sole Ca_{2+}-permeable current activated by IP3-dependent depletion of the ER Ca_{2+} store. Electrophysiological recordings carried out on many different cell types revealed the existence of a store-operated current, termed Store-Operated Activated Calcium Current (I_{SOC}), which exhibited slightly biophysical properties as respect to the I_{CRAC} [29]. The I_{SOC} is generally permeable to Ca_{2+}, Na_{2+}, K_{+} and Cs_{+} and exhibits a significantly greater conductance than Orai1 channels, ranging from 1 to 43 pS [29]. The I_{SOC} is mediated by members of the TRPC sub-family of non-selective
cation channels, which are activated as a consequence of PLC stimulation [72]. TRPC channels are subdivided into four subsets based on their sequence homology: TRPC1, TRPC2, which is a pseudogene in humans, TRPC4/5, and TRPC3/6/7 [73]. Typically, TRPC channels are present in the plasma membrane or in specialized lipid microdomains containing caveolae [74,75]. All members of the TRPC family share a common topology [76]. The cytoplasmic N- and C-termini are separated by six transmembrane domains (TM1-TM6), including a putative pore region (LFW pore motif) between TM5 and TM6 [77,78]. The N-terminus is composed of three to four ankyrin repeats, a predicted coiled-coil region, a putative caveolin binding region and a Protein Kinase G (PKG) phosphorylation sites. The ankyrin repeats and the caveolin binding region appear to be required for correct targeting of TRPC to the plasma membrane [79], while the coiled-coil motif plays a role in the control of TRPC oligomerisation [80]. The cytoplasmic C-terminus includes a Protein Kinase C (PKC) phosphorylation sites, the TRP signature motif (EWKFAR), a highly conserved proline-rich motif, a predicted coiled-coil region and the CIRB (calmodulin/IP3 receptor binding) region which may contribute to plasma membrane targeting [76,81–85]. TRPC channels may assemble in both homomeric and heteromeric complexes, thereby giving rise to a bewildering variety of cationic channels whose biophysical features and physiological roles are yet to be fully dissected. For instance, TRPC1 may associate with TRPC4 and TRPC5, while TRPC3 has the potential to interact with TRPC3 and TRPC7 [77,78,86]. Furthermore, they can form heterometic channel complexes with other members of the TRP super-family. For instance, TRPC1 has been shown to associate with either Transient Receptor Potential Vanilloid 4 (TRPV4) [87], TRPV6 [88], or the Transient Receptor Potential Ankyrin-1 (TRPA1) [89]. As regard to SOCE, all TRPC channels have been associated to the $I_{\text{SOC}}$ developing in response to ER Ca$^{2+}$ depletion [90,91]. As discussed elsewhere [90,91], however, the strongest evidence in favor of TRPC channel contribution to SOCE has been provided for TRPC1 [92–94] and TRPC4 [95–97], while the operation-mode of TRPC3 depends on its expression levels in naïve tissues [98] and TRPCs 5, 6, and 7 serve as receptor-operated channels [99]. The store-sensitivity of TRPC1 and TRPC4 depends on their ability to bind to STIM1. Earlier studies showed that gating of TRPC1 can be gated by the electrostatic interaction between the negatively charged aspartate residues in TRPC1 (639DD640) with the positively charged lysines in the STIM1 K-domain (684KK685) [100]. These acidic residues are conserved among all TRPC channels and underlies also STIM1-dependent TRPC4 activation following InsP$_3$-dependent Ca$^{2+}$ release [101]. The exact mechanism that determines which TRPC isoforms are recruited by STIM1 upon depletion of the ER Ca$^{2+}$ pool remains elusive, but could involve either their localization at precise sites within the plasma membrane, such as caveolae, or their propensity to interact with Orai1 [90,99]. Intriguingly, several reports revealed that knocking down Orai1 suppressed SOCE despite the presence of endogenous or heterologously expressed STIM1 and TRPC1. STIM1, Orai1 and TRPC1 were found to associate into a heteromeric supermolecular complex in response to ER Ca$^{2+}$ depletion in many different cell types, including in human salivary gland (HSG) cell [102], human platelets [103], human liver cells [104], mouse pulmonary arterial smooth muscle cells [105], human parathyroid cells [106], and rat kidney fibroblasts [107]. Several models have been proposed to interpret this interaction [90,99,108,109]. Orai1 and TRPC1 could both contribute to line the channel pore, each being activated by STIM1 upon store depletion. Alternatively, Orai1 has been suggested to mediate the STIM1-dependent activation of TRPC1, which, in this scenario, would contribute the pore-forming subunit of the store-operated channel [103]. More recently, Ambudkar’s group showed that Orai1 and TRPC1 form distinct STIM1-regulated Ca$^{2+}$-permeable channels. Studies conducted on HSG cells unveiled that Orai1-dependent Ca$^{2+}$ entry results in TRPC1 recruitment to the plasma membrane in close proximity to Orai1; herein, TRPC1 is subsequently gated by STIM1 [110]. As a consequence, the $I_{\text{SOC}}$ recorded in these cells is a mixed current composed by the TRPC1/STIM1-mediated non-selective cation current flowing through TRPC1 and by the Orai1/STIM1-mediated $I_{\text{CRAC}}$ [90,110]. This mechanism would also explain why STIM1, Orai1 and TRPC1 co-immunoprecipitate upon ER Ca$^{2+}$ depletion in the cellular models described above.
4. Thrombopoiesis: The Long Route of Megakaryocytes to Platelet Production

In adult mammals, hematopoiesis occurs in the bone marrow, which supports simultaneously the life-long maintenance of hematopoietic stem cells (HSCs) and the regulated production of end-stage lymphoid, myeloid and erythroid cells [111]. Thrombopoiesis is defined as the process by which mature megakaryocytes are derived from HSCs to produce platelets [112,113], the smallest cells in the human blood (≈3 µm), which perform crucial roles in hemostasis, but also in several other processes such as angiogenesis, immunity, tissue regeneration and wound healing [114].

The first step of megakaryocyte development is regulated by the lineage-specific growth factor Thrombopoietin (TPO) [115] and consists in HSC commitment with arrest of proliferation and initiation of endomitosis, the process by which megakaryocytes increase their nuclear content developing polyploid multilobed nuclei [116,117]. The second step is associated with cytoplasm expansion and intense synthesis of proteins to be delivered into the secretory granules. The most abundant being α-granules, containing Platelet Factor 4 (PF4), von Willebrand Factor (vWF), fibronectin, Platelet Derived Growth Factor (PDGF), Vascular Endothelial Growth Factor (VEGF) and Transforming Growth Factor-β1 (TGF-β1), and δ-granules, enriched with small molecules such as serotonin, epinephrine, adenosine triphosphate (ATP), ADP and ions [118–120]. Once mature, megakaryocytes come in close contact with bone marrow sinusoids, under Stromal Derived Factor-1α (SDF-1α) chemo-attraction, and they undergo characteristic changes in cytoskeleton structure with the extension of multiple long pseudopods, called proplatelets, that assemble nascent platelet at their terminal ends [117,121,122]. Finally, proplatelets extend, through the vascular endothelium, into the lumen of sinusoidal vessels, where the release of mature platelets can be attributed to blood hydrodynamics which allow their shedding form the proplatelet shaft, as demonstrated in vivo in mice by multiphoton intravital microscopy [123,124], and confirmed ex vivo in humans, by employing a variety of cell culture techniques and biomimetic platforms reproducing human platelet release [125–128]. During its lifespan, a mature megakaryocyte can produce up to 10⁴ platelets and each day a human adult produces 10¹¹ platelets, a number that can increase in response to acute platelet demand by rapid fragmentation of megakaryocyte cytoplasm [129,130].

The production of platelets is a complex process that involves the support of both bone marrow extracellular matrix (ECM) components and soluble factors. ECMs represent the main bone marrow scaffolding, which surrounds islets of HSCs and committed hematopoietic progenitors [131–133]. Within this microenvironment, while differentiating, megakaryocytes may encounter ECMs that differently regulate thrombopoiesis. For instance, it has been described that type I collagen prevents premature platelet release, while supporting megakaryocyte motility [134,135]; while fibronectin and type IV collagen sustain proplatelet formation, but also contribute to the regulation of cell proliferation and differentiation [136,137]. It is known that ECM components are produced by bone marrow stromal cells [138–140], however we recently demonstrated that both mouse and human megakaryocytes can actively synthesize and deposit collagens and fibronectin [137,141]. In particular, we showed that TPO is a pivotal regulator of this function by inducing TGF-β1 release, thus controlling ECM component synthesis in an autocrine manner. TGF-β1, as well as other soluble factors (e.g., ADP, VEGF, PF4) and ECM components (e.g., vWF, fibronectin), have been shown to be constitutively released by megakaryocytes to regulate their own differentiation and proplatelet formation [134,136,142–146], indicating that in physiological conditions megakaryocytes can activate an autocrine/paracrine loop which contribute to both their own development and overall bone marrow homeostasis [147]. Consistently, impaired synthesis and release of these proteins has been linked to altered megakaryocyte maturation, proplatelet formation and/or ECMs production [141–143,148], leading to a broad spectrum of clinical outcomes, from defective peripheral blood platelet count to deregulated bone marrow homeostasis [147,149,150].

5. Biogenesis of Store-Operated Calcium Entry during Thrombopoiesis: Biological Significance in Physiology and Pathology

Recent progresses revealed that most of the signals that regulate platelet production converge into the regulation of the expression and/or activation of SOCE, thus suggesting that Ca²⁺ may
have the ability to decode the massages from multiple complex and dynamic inputs ad covert
them into a single response having, as major effect, the control of the ordinary course of platelet
production. As a consequence, as we will now discuss, impairment of this function may results in
pathological phenotypes.

5.1. Development of Endoplasmic Reticulum and Endoplasmic Reticulum-Related Proteins in Megakaryocytes

During commitment megakaryocytes can be assigned to distinct stages of maturity according
to standard morphological criteria [151]. Specifically, in the early maturation stage, megakaryocytes
usually present the lowest cytoplasmic/nuclear ratio, compact nucleus and small size, with fewer
and undeveloped non-specific membranous organelles, such as mitochondria, Golgi apparatus,
and smooth and rough ER. In the successive stages a progressive cytoplasmic mass increase and
appearance of highly lobulated nuclei are accompanied by the expansion of all organelles, especially
of Golgi apparatus and ER, which contribute to a continuous membrane supply for the growth of
the demarcation membrane system (DMS), an extensive system that provide a membrane reservoir
for the formation of future platelets [152,153]. The ER plays primarily a key role in regulating Ca

++
signaling through SERCA and IP3Rs. Interestingly, Lacabaratz-Porret et al. analyzed the ER-protein
patterns during thrombopoiesis and demonstrated that TPO stimulates the expression and synthesis
of SERCA3 throughout megakaryocyte maturation, while SERCA2b is constitutively expressed by
megakaryocytes [154]. Importantly, a specific increase in the expression of SERCA3a has been
confirmed also in in vitro differentiated human megakaryocytes during proplatelet formation [155].
Further, the presence of IP3R types I, II and III have been shown in the megakaryocytic cell, with the
expression of IP3R II and III being slightly up-regulated upon differentiating stimulus [154], consistent
with previous findings demonstrating that the expression profile of IP3R subtypes is dynamically
modified during hematopoiesis depending on the stimuli that induce differentiation [156]. Therefore,
a profound reorganization of the ER and of ER-proteins, involved in promoting store emptying, is
achieved during late stages of thrombopoiesis.

5.2. Expression and Function of Transient Receptor Potential Canonical during Megakaryocyte Differentiation

Since STIMs and Orai have been discovered and characterized later than TRPCs, early evidence
that cells belonging from the megakaryocytic lineage could express genes of the SOCE family was first
given in 1997 by Berg et al. [157]. At that time, three human TRPC genes, TRPC1, TRPC2 and TRPC3,
were shown to be present in different human megakaryocyte cell lines (MEG01, DAMI and HEL), thus
suggesting the involvement of TRPCs in regulating Ca
++
homeostasis in these cells [157]. These data
were confirmed and extended by Wakabayashi et al. who observed SOCE activation in megakaryocytic
cell lines, utilizing thapsigargin as a tool to accomplish store depletion, and hypothesized a role for
TRPC4 as the molecular component that determines the sensitivity of store-operated channels to
intracellular alkalosis in this lineage [158]. The first evidence that primary megakaryocytes express
functional SOCE was given by den Dekker et al., who demonstrated the presence of unspliced
isoforms for TRPC1, 4 and 6 in human immature (Cluster of differentiation (CD)61/CD42b
low
) and
mature (CD61/CD42b
high
) megakaryocytes, differentiated in vitro from human cord blood derived
CD34+
HSCs [159]. Interestingly, the same group observed high Ca
++
influx in megakaryocytes, either upon store depletion by thapsigargin or by the receptor agonist thrombin, in both immature
and mature megakaryocytes, thus demonstrating functional activity of SOCE early during the
lineage specification [160]. Moreover, they observed an increase in Gqα and Gia1/2 expression
in maturing human megakaryocytes, which was accompanied by an increase in intracellular Ca
++
signals triggered not only by physiological agonists, such as thrombin and ADP, but also by TPO [160].
Of note, TPO-induced Ca
++
signal changed from a single peak in immature megakaryocytes into
a series of oscillatory Ca
++
spikes in mature cells. It is known that the function of the oscillatory
discharges of Ca
++
is to produce a sufficiently ample drop in ER Ca
++
levels to activate SOCE,
which provides the necessary Ca
++
influx capable of providing localized signals that specifically
couple to downstream effector pathways regulating cellular functions [161–163]. For instance, Ca\(^{2+}\) oscillations could confer cell survival and drive differentiation in different cell types [164–167]. Therefore, TPO-induced Ca\(^{2+}\) signaling may be involved in modulation of megakaryopoiesis. To this regard, more recently, Ramanathan and Mannhalter showed that TRPC6 increases form early stages of megakaryocytic commitment to mature megakaryocytes to regulate TPO-induced cell proliferation through a store-independent Ca\(^{2+}\) entry pathway [168], indicating that a combined effort of SOCE and non-SOCE TRPC channels is involved in regulating Ca\(^{2+}\) flows in megakaryocytes. The expression of TRPC1 and TRPC6 was also confirmed in murine megakaryocytes [169]. Specifically, the individual selection of these cells directly from bone marrow specimens allowed the demonstration of a major role for TRPC6 in allowing Ca\(^{2+}\) influx upon physiological stimulation with ADP. However, TRPC1\(^{-/-}\) and TRPC6\(^{-/-}\) mice did not show defective thrombopoiesis as indicated by normal platelet count and size in the peripheral blood [170,171]. Noteworthy, the evidence that megakaryocytes express different isoforms of other store-operated channels, make it difficult to believe that a single knockdown may significantly affect megakaryocyte function.

5.3. NF-κB Pathway Is a Major Regulator of Orai Expression in Megakaryocytes

An important next-step toward a better comprehension of the role of SOCE in megakaryocyte physiology was made after the identification of STIM and Orai families as major determinant of SOCE. Since that, Orai1 expression was observed in the human megakaryocytic cell line MEG-01 [172,173]. Borst et al. demonstrated that Orai1 expression is modulated by the serum- and glucocorticoid-inducible kinase 1 (SGK1) [172], a kinase belonging to the AGC family of serine/threonine protein kinases [174,175], that can be regulated by a variety of different triggers, including hormones, thrombin, oxidative stress and growth factors [175]. Specifically, they showed that SGK1 regulates Orai1 expression in megakaryocytes through a nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) dependent pathway which in turn ensures physiological thrombopoiesis, whereas platelet form sgrk1\(^{-/-}\) mice displayed a significantly blunted SOCE and agonist-induced increased [Ca\(^{2+}\)]\(_i\) resulting in impaired platelet activation [172]. Further evidence about the relevance of this pathway was provided by the observation that megakaryocytic cell transfection with the NF-κB p50/p65 heterodimer significantly increases STIM1/Orai1 transcription and protein levels, while 1,25(OH)\(_2\) vitamin D\(_3\) decreases STIM1/Orai1 expression and I\(_{CRAC}\) in megakaryocyte by negatively modulating NF-κB activity [176]. Moreover, TGF-β1, that is increasingly released during megakaryocyte differentiation regulating platelet production [142], has been recently shown to be a stimulator of SOCE in megakaryocytes, via the up-regulation of SGK1, which in turn activates nuclear factor NF-κB and stimulates Orai1 expression [177]. Interestingly, Almilaji et al. demonstrated that TGF-β1 significantly up-regulates also Na\(^+\)/Ca\(^{2+}\)-exchanger activity in murine bone marrow megakaryocytes, thus influencing megakaryocytic Ca\(^{2+}\) signaling not only by augmenting Ca\(^{2+}\) entry, but also by stimulating Ca\(^{2+}\) extrusion [178].

5.4. Store-Operated Calcium Entry Finely Regulates Physiological Megakaryocyte Functions

The activity of the molecular effectors of SOCE has been shown to be finely regulated during megakaryocyte differentiation. Particularly, Albarrán et al. demonstrated that the TRPA1, a negative regulator of STIM1 and Orai1 interaction, is down-regulated in late phases of megakaryocyte differentiation in order to confer enhanced SOCE functionality to mature megakaryocytes and released platelet particles [89]. The relevance of this finding was clarified in 2014 when our group investigated for the first time the mechanistic link between Ca\(^{2+}\) signaling and thrombopoiesis [28]. Specifically, we showed that in vitro differentiated megakaryocytes, from human cord blood derived CD34\(^+\) HSCs, express all the molecular candidates to mediate SOCE, including STIM1, Orai1, and TRPC1, and that pharmacological-induced intracellular Ca\(^{2+}\) release from ER by cyclopiazonic acid promotes their active interaction and consequent extracellular Ca\(^{2+}\) flow inside cell cytoplasm [28]. In this context, different functional and biochemical assays evidenced a compartmentalized distinct role of
the two Ca\textsuperscript{2+} release/entry routes in response to ADP [28], an autocrine modulator of proplatelet formation [143,179]. Specifically, IP3-dependent Ca\textsuperscript{2+} mobilization from intracellular stores was primarily involved in the activation of biochemical signaling cascades (e.g., Akt, Erk1/2) that promote proplatelet formation, while extracellular Ca\textsuperscript{2+} entry was mainly responsible for the regulation of contractile forces that favors megakaryocyte sensing of extracellular substrates and motility in adhesion to different ECM components (e.g., fibronectin and type I collagen) [28]. In the light of the previous demonstration that human mature megakaryocytes display a significant increase in [Ca\textsuperscript{2+}]\textsubscript{i} upon collagen stimulation [180], these findings support the involvement of SOCE in regulating megakaryocyte interaction with the bone marrow microenvironment, that in turn support proliferation and platelet production [137]. Interestingly, Ca\textsuperscript{2+} entry in megakaryocytes in response to ADP can be amplified by glutamate-induced activation of ionotropic N-methyl-D-aspartate receptors (NMDARs), resulting in increased cell proliferation, rather than differentiation, while NMDAR antagonists reduce cell growth and promote differentiation of leukemic megakaryoblasts [181].

All together, these data suggest that a tight control of [Ca\textsuperscript{2+}]\textsubscript{i} is achieved by megakaryocytes along their route to platelet production by different stimuli that integrate in order to ensure the fine balance between cell proliferation and differentiation.

5.5. Over-Activated Store-Operated Calcium Entry Is Observed in Pathological Thrombopoiesis

A pronounced megakaryocyte hyperplasia has been shown in heterozygous mice expressing an activating EF hand mutant of STIM1 (STIM1\textsuperscript{Sax/+}), resulting in the constitutive activation of SOCE and consequent high cytoplasmic Ca\textsuperscript{2+} levels [182]. Macrothrombocytopenia and an associated bleeding disorder were also observed in these mice, due to high basal intracellular Ca\textsuperscript{2+} levels in circulating platelets responsible for a pre-activation state and consequent increased platelet consumption [182], further supporting the evidence that a fine regulation of SOCE in maturing megakaryocyte is crucial to ensure physiological thrombopoiesis. Of note, the megakaryocytic hyperplasia in STIM1\textsuperscript{Sax/+} mice was accompanied, at the age of 6 months, by the appearance of bone marrow fibrosis and severe splenomegaly [182], all symptoms resembling characteristic features of Primary Myelofibrosis (PMF), an hematopoietic malignancy belonging to the family of Philadelphia-negative Myeloproliferative Neoplasms (MPNs) [183,184]. It is known that patients affected by PMF present increased deposition of ECM components within bone marrow, resulting in a progressive fibrosis that alters the bone marrow microenvironment architecture, compromising efficient platelet production and overall hematopoiesis, which moves into the spleen leading to splenomegaly, further worsening the prognosis of affected patients [185]. Interestingly, bone marrow megakaryocytes are considered key players in the PMF pathogenesis because of the alteration of both their number and morphology (hyperplasia and dysplasia) [142,186,187]. Further, proplatelet formation in PMF has been described to present several structural alterations, and platelet count may vary from low to abnormally high numbers, with thrombosis as main additional cause of reduced patient survival [186,188]. Importantly, progression to myelofibrosis (secondary myelofibrosis) may occur also in patients affected by Essential Thrombocythemia (ET), another MPN characterized by megakaryocytic hyperplasia and elevated platelet count.

Both PMF end ET are caused in ~10% of cases by mutations of MPL gene, leading to a constitutive activation of c-Mpl, the TPO receptor, and in other ~60% by mutations in JAK2 gene, resulting in the constitutive activation of its signaling pathway which is normally activated downstream of c-Mpl upon TPO stimulation [188]. Recent findings shed new light in the genetic origin of MPNs by the description of additional somatic mutations in CALR, the gene encoding for the ER chaperone calreticulin, which was found in ~25% of patients with MPL or JAK2 unmutated disease [189,190]. As for MPL and JAK2 gene mutations, megakaryocytes expressing the mutated calreticulin present constitutive stimulation of c-Mpl downstream signaling, due to an unexpected activating interaction between the receptor and the mutated protein [191,192]. Calreticulin is a multifunctional protein that normally participates in ER Ca\textsuperscript{2+} storage and buffering [193]. All the mutations that have been described affect a region of the gene that encodes for the C-terminal peptide, with the resulting mutant proteins sharing a novel amino acid
sequence containing positively charged amino acids, whereas the non-mutant protein is largely negatively charged [194]. In particular, a 52-base pair (bp) deletion (type-1) and a 5-bp insertion (type-2 mutation) are the most frequent variants. The discovery of these mutations triggered research at investigating the changes, if any, in Ca\(^{2+}\) homeostasis in megakaryocytes harboring the different mutations. To this regard, we demonstrated that the type-1 mutation enhanced both ER-dependent intracellular Ca\(^{2+}\) release and SOCE with respect to healthy controls, and JAK2 or type-2 mutated megakaryocytes [194]. Whether the higher SOCE magnitude could be due to the higher ER Ca\(^{2+}\) mobilization or to the end tail modifications of calreticulin mutants still remains to be elucidated. The higher intraluminal Ca\(^{2+}\) discharge is in line with the notion that, in type-1 mutation, the negatively charged amminoacids responsible for Ca\(^{2+}\) binding at the C-terminal are almost entirely replaced by either positively charged or neutral residues, while type-2 mutation does not result in the loss of stretch II and III [194]. Intriguingly, several studies reported that overexpression of calreticulin attenuated SOCE in various cell types and that this effect was likely to be mediated by the negatively charged carboxyl-terminal domain [195–197], thus suggesting that type-1 mutation could impair somehow SOCE machinery. Type-1 mutations are mainly associated with a significantly higher risk of myelofibrotic transformation, while type-2 mutations are preferentially associated with an indolent clinical course [194]. Based on the current knowledge about the role of SOCE in megakaryocyte physiology, we can hypothesize that high Ca\(^{2+}\) entry may have a major impact in promoting cell proliferation and migration, thus resulting in increased platelet release, justifying the augmented platelet count observed in these patients (Figure 1). Further, it has been recently described that, aside from their ability to form platelets, megakaryocytes participate to bone marrow homeostasis by releasing growth factors, such as TGF-\(\beta\)1, that influence ECM components deposition by both stromal cells and megakaryocytes themselves [141]. However, to which extent SOCE and mutated calreticulin may contribute to TGF-\(\beta\)1 and/or matrix deposition by diseased megakaryocytes remains to be clarified.

**Figure 1.** Store-Operated Ca\(^{2+}\) Entry (SOCE) in physiological and pathological thrombopoiesis. (A) Bone marrow, contained in spongy bones, is a tridimensional network of branching sinusoids surrounding islets of hematopoietic cells. Within this environment hematopoietic stem cells (HSCs) undergo self-renewal as well as differentiation into committed lineages in order to support the physiological homeostasis of all blood cells. Megakaryopoiesis takes place under thrombopoietin stimulation, which promotes HSC commitment and differentiation toward megakaryocytes (MKs). In MKs with replete endoplasmic reticulum (ER), Stromal Interaction Molecule (STIM) is localized in an inactive configuration in the ER membrane. Depletion of Ca\(^{2+}\) stores triggers Ca\(^{2+}\) release from the ER through inositol-trisphosphate receptors (IP3R) and consequent Ca\(^{2+}\) dissociation form STIM, which oligomerize and translocate next to the plasma membrane. Then, STIM binding to Orai and Transient Receptor Potential Canonical (TRPC) results in opening of these channels and extracellular Ca\(^{2+}\) entry. The increased cytosolic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_{i}\)) in turn regulates cell proliferation, differentiation, migration and final platelet production; (B) An abnormal increase in [Ca\(^{2+}\)]\(_{i}\), due to altered control of SOCE dynamics may result in pathological phenotypes such as higher proliferation and platelet production. Red arrow, increased with respect to physiological conditions.
6. Conclusions

After 30 years from the first description of capacitative Ca\textsuperscript{2+} entry, many researches have been devoted to identify molecular and mechanistic process related to SOCE. In this review, we have summarized the whole machinery of SOCE, which involve STIMs, Orais and TRPCs in an elegant choreography that connects intracellular Ca\textsuperscript{2+} release to plasma membrane channels promoting extracellular Ca\textsuperscript{2+} entry. In megakaryocytes SOCE regulates fundamental cellular functions, such as proliferation, migration and sensing of extracellular environment. However, little is still known about the specific molecular and biochemical signals that are targeted by Ca\textsuperscript{2+} in order to drive thrombopoiesis. Understanding in details the exact mechanisms by which SOCE control megakaryocyte functions would be instrumental for the identification of novel pathways that may be involved in the pathogenesis of megakaryocyte-related diseases.

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Abbreviations

| Term             | Definition                                                                 |
|------------------|-----------------------------------------------------------------------------|
| ADP              | Adenosine diphosphate                                                       |
| ATP              | Adenosine triphosphate                                                      |
| Ca\textsuperscript{2+} | Calcium                                                                     |
| [Ca\textsuperscript{2+}]\textsubscript{i} | Cytoplasmic calcium concentration                                           |
| CALR             | Calreticulin                                                                |
| CC               | Coiled-coil                                                                 |
| CD               | Cluster of differentiation                                                  |
| CDI              | Calcium-dependent inactivation                                              |
| cEF              | Canonical EF-hand domain                                                     |
| CIRB             | Calmodulin/inositol 1,4,5-trisphosphate receptor binding region              |
| CRAC             | Calcium-release activated calcium channel                                   |
| Cs\textsuperscript{+} | Cesium                                                                     |
| c-Mpl            | Thrombopoietin receptor                                                      |
| DAG              | Diacylglycerol                                                              |
| DMS              | Demarcation membrane system                                                 |
| dOrai            | Drosofila Orai                                                              |
| ECM              | Extracellular matrix                                                        |
| ET               | Essential thrombocytemia                                                    |
| GPCR             | G-protein coupled receptor                                                  |
| hEF              | Hidden non-canonical EF-hand domain                                          |
| HSC              | Hematopoietic stem cells                                                    |
| HSG              | Human salivary gland                                                        |
| I\textsubscript{CRAC} | Calcium release-activated calcium current                                   |
| IP3              | Inositol 1,4,5-trisphosphate                                                |
| IP3R             | Inositol 1,4,5-trisphosphate receptor                                        |
| I\textsubscript{SOC} | Store-operated activated calcium current                                    |
| JAK2             | Janus kinase 2                                                              |
| K\textsuperscript{+} | Potassium                                                                  |
| MPN              | Philadelphia-negative myeloproliferative neoplasm                           |
| Na\textsuperscript{+} | Sodium                                                                     |
| NCX              | Sodium/calcium exchanger                                                    |
| E\textsubscript{rev} | Reversal potential                                                         |
| NF-kB            | Nuclear factor κ-light-chain-enhancer of activated B cells                  |
| NMDAR            | Ionotropic N-methyl-D-aspartate receptor                                     |
| PDGF             | Platelet derived growth factor                                              |
PF4 Platelet factor 4
PF2 Phosphatidylinositol 4,5-bisphosphate
PKC Protein kinase C
PKG Protein kinase G
PLC Phospholipase C
PMCA Plasma membrane calcium-ATPase
PMF Primary myelofibrosis
RNA Ribonucleic acid
ROC Receptor-operated channel
RyR Ryanodine receptors
SAM Sterile-α motif
SDF-1α Stromal derived factor-1α
SERCA Sarco/endoplasmic reticulum calcium-ATPase
SGK1 Serum- and glucocorticoid-inducible kinase 1
SMOC Second messenger-operated channel
SOC Store-operated channel
SOCE Store-operated calcium entry
SR/ER Sarco/endoplasmic reticulum
TGF-β1 Transforming growth factor-β1
TKR Tyrosine kinase receptor
TM Transmembrane
TPO Thrombopoietin
TRPA-1 Transient receptor potential ankyrin-1
TRPC Transient receptor potential canonical
TRPV Transient receptor potential vanilloid
VEGF Vascular endothelial growth factor
VOC Voltage-operated channel
vWF Von Willebrand Factor

References
1. Carafoli, E. Calcium signaling: A tale for all seasons. Proc. Natl. Acad. Sci. USA 2002, 99, 1115–1122. [CrossRef] [PubMed]
2. Berridge, M.J.; Lipp, P.; Bootman, M.D. The versatility and universality of calcium signalling. Nat. Rev. Mol. Cell Biol. 2000, 1, 11–21. [CrossRef] [PubMed]
3. Berridge, M.J.; Bootman, M.D.; Roderick, H.L. Calcium signalling: Dynamics, homeostasis and remodelling. Nat. Rev. Mol. Cell Biol. 2003, 4, 517–529. [CrossRef] [PubMed]
4. Pozzan, T.; Rizzuto, R.; Volpe, P.; Meldolesi, J. Molecular and cellular physiology of intracellular calcium stores. Physiol. Rev. 1994, 74, 595–636. [PubMed]
5. Sorrentino, V.; Rizzuto, R. Molecular genetics of Ca2+ stores and intracellular Ca2+ signalling. Trends Pharmacol. Sci. 2001, 22, 459–464. [CrossRef]
6. Lim, D.; Bertoli, A.; Sorgato, M.C.; Moccia, F. Generation and usage of aequorin lentiviral vectors for Ca2+ measurement in sub-cellular compartments of hard-to-transfect cells. Cell Calcium 2016, 59, 228–239. [CrossRef] [PubMed]
7. Clapham, D.E. Calcium signaling. Cell 1995, 80, 259–268. [CrossRef]
8. Moccia, F.; Nusco, G.A.; Lim, D.; Ercolano, E.; Gragnaniello, G.; Brown, E.R.; Santella, L. Ca2+ signalling and membrane current activated by cADPr in starfish oocytes. Pflugers Arch. 2003, 446, 541–552. [CrossRef] [PubMed]
9. Ronco, V.; Potenza, D.M.; Denti, F.; Vullo, S.; Gagliano, G.; Tognolina, M.; Guerra, G.; Pinton, P.; Genazzani, A.A.; Mapelli, L.; et al. A novel Ca2+-mediated cross-talk between endoplasmic reticulum and acidic organelles: Implications for NAADP-dependent Ca2+ signalling. Cell Calcium 2015, 57, 89–100. [CrossRef] [PubMed]
10. Prakriya, M.; Lewis, R.S. Store-operated calcium channels. Physiol. Rev. 2015, 95, 1383–1436. [CrossRef] [PubMed]
11. Hooper, R.; Rothberg, B.S.; Soboloff, J. Neuronal stimulation at rest. Sci. Signal. 2014, 7, pe18. [CrossRef] [PubMed]
12. Moccia, F.; Zuccolo, E.; Soda, T.; Tanzi, F.; Guerra, G.; Mapelli, L.; Lodola, F.; D’Angelo, E. STIM and Orai proteins in neuronal Ca2+ signaling and excitability. Front. Cell. Neurosci. 2015, 9, 153. [CrossRef] [PubMed]
Feske, S.; Gwack, Y.; Prakriya, M.; Srikanth, S.; Puppel, S.H.; Tanasa, B.; Hogan, P.G.; Lewis, R.S.; Daly, M.; Roos, J.; DiGregorio, P.J.; Yeromin, A.V.; Ohlsen, K.; Lioudyno, M.; Zhang, S.; Safrina, O.; Kozak, J.A.; Stiber, J.; Hawkins, A.; Zhang, Z.S.; Wang, S.; Burch, J.; Graham, V.; Ward, C.C.; Seth, M.; Finch, E.; Locke, E.G.; Bonilla, M.; Liang, L.; Takita, Y.; Cunningham, K.W.

A homolog of voltage-gated Ca^{2+} channels stimulated by depletion of secretory Ca^{2+} in yeast. Mol. Cell. Biol. 2000, 20, 6686–6694. [CrossRef] [PubMed]

Wes, P.D.; Chevesich, J.; Jeromin, A.; Rosenberg, C.; Stetten, G.; Montell, C. TRPC1, a human homolog of a Drosophila store-operated channel. Proc. Natl. Acad. Sci. USA 1995, 92, 9652–9656. [CrossRef] [PubMed]

Putney, J.W. A model for receptor-regulated calcium entry. Cell Calcium 1986, 7, 1–12. [CrossRef]

Takemura, H.; Putney, J.W. Capacitative calcium entry in parotid acinar cells. Biochem. J. 1989, 258, 409–412. [CrossRef] [PubMed]

Feske, S. Immunodeficiency due to defects in store-operated calcium entry. Ann. N. Y. Acad. Sci. 2011, 1238, 74–90. [CrossRef] [PubMed]

Jang, S.I.; Ong, H.L.; Liu, X.; Alevizos, I.; Ambudkar, I.S. Up-regulation of store-operated Ca^{2+} entry and nuclear factor of activated T cells promote the acinar phenotype of the primary human salivary gland cells. J. Biol. Chem. 2016, 291, 8709–8720. [CrossRef] [PubMed]

Toth, A.B.; Shum, A.K.; Prakriya, M. Regulation of neurogenesis by calcium signaling. Cell Calcium 2016, 59, 124–134. [CrossRef] [PubMed]

Moccia, F.; Dragoni, S.; Lodola, F.; Bonetti, E.; Bottino, C.; Guerra, G.; Laforenza, U.; Rosti, V.; Tanzi, F. Store-dependent Ca^{2+} entry in endothelial progenitor cells as a perspective tool to enhance cell-based therapy and adverse tumour vascularization. Curr. Med. Chem. 2012, 19, 5802–5818. [CrossRef] [PubMed]

Stiber, J.; Hawkins, A.; Zhang, Z.S.; Wang, S.; Burch, J.; Graham, V.; Ward, C.C.; Seth, M.; Finch, E.; Malouf, N.; et al. STIM1 signalling controls store-operated calcium entry required for development and contractile function in skeletal muscle. Nat. Cell Biol. 2008, 10, 688–697. [CrossRef] [PubMed]

Berra-Romani, R.; Mazzocco-Spezzia, A.; Pulina, M.V.; Golovina, V.A. Ca^{2+} handling is altered when arterial myocytes progress from a contractile to a proliferative phenotype in culture. Am. J. Physiol. Cell Physiol. 2008, 295, C779–C790. [CrossRef] [PubMed]

Potier, M.; Gonzalez, J.C.; Motiani, R.K.; Abdullaev, LF; Bisaillon, J.M.; Singer, H.A.; Trebak, M. Evidence for STIM1- and Orai1-dependent store-operated calcium influx through I_{CRAC} in vascular smooth muscle cells: Role in proliferation and migration. FASEB J. 2009, 23, 2425–2437. [CrossRef] [PubMed]

Collins, H.E.; Zhu-Mauldin, X.; Marchase, R.B.; Chatham, J.C. STIM1/Orai1-mediated SOCE: Current perspectives and potential roles in cardiac function and pathology. Am. J. Physiol. Heart Circ. Physiol. 2013, 305, H446–H458. [CrossRef] [PubMed]

Courjaret, R.; Machaca, K. STIM and Orai in cellular proliferation and division. Front. Biosci. 2012, 4, 331–341. [CrossRef]

Kar, P.; Parekh, A. STIM proteins, Orai1 and gene expression. Channels 2013, 7, 374–378. [CrossRef] [PubMed]

Di Buduo, C.A.; Moccia, F.; Battiston, M.; de Marco, L.; Mazzuccato, M.; Moratti, R.; Tanzi, F.; Balduin, A. The importance of calcium in the regulation of megakaryocyte function. Haematologica 2014, 99, 769–778. [CrossRef] [PubMed]

Parekh, A.B.; Putney, J.W. Store-operated calcium channels. Physiol. Rev. 2005, 85, 757–810. [CrossRef] [PubMed]

Hoth, M.; Penner, R. Depletion of intracellular calcium stores activates a calcium current in mast cells. Nature 1992, 355, 353–356. [CrossRef] [PubMed]

Penner, R.; Fasolato, C.; Hoth, M. Calcium influx and its control by calcium release. Curr. Opin. Neurobiol. 1993, 3, 368–374. [CrossRef]

Liu, J.; Kim, M.L.; Heo, W.D.; Jones, J.T.; Myers, J.W.; Ferrell, J.E.; Meyer, T. STIM is a Ca^{2+} sensor essential for Ca^{2+}-store-depletion-triggered Ca^{2+} influx. Curr. Biol. 2005, 15, 1235–1241. [CrossRef] [PubMed]

Roos, J.; DiGregorio, P.J.; Yeromin, A.V.; Ohlsen, K.; Lioudyno, M.; Zhang, S.; Safrina, O.; Kozak, J.A.; Wagner, S.L.; Cahalan, M.D.; et al. STIM1, an essential and conserved component of store-operated Ca^{2+} channel function. J. Cell Biol. 2005, 169, 435–445. [CrossRef] [PubMed]

Feske, S.; Gwack, Y.; Prakriya, M.; Srikanta, S.; Puppel, S.H.; Tanasa, B.; Hogan, P.G.; Lewis, R.S.; Daly, M.; Rao, A. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. Nature 2006, 441, 179–185. [CrossRef] [PubMed]
35. Zhang, S.L.; Yeromin, A.V.; Zhang, X.H.; Yu, Y.; Safrina, O.; Penna, A.; Roos, J.; Stauderman, K.A.; Cahalan, M.D. Genome-wide RNAi screen of Ca\textsuperscript{2+} influx identifies genes that regulate Ca\textsuperscript{2+} release-activated Ca\textsuperscript{2+} channel activity. Proc. Natl. Acad. Sci. USA 2006, 103, 9357–9362. [CrossRef] [PubMed]

36. Zhang, S.L.; Yu, Y.; Roos, J.; Kozak, J.A.; Deerinck, T.J.; Ellisman, M.H.; Stauderman, K.A.; Cahalan, M.D. STIM1 is a Ca\textsuperscript{2+} sensor that activates CRAC channels and migrates from the Ca\textsuperscript{2+} store to the plasma membrane. Nature 2005, 437, 902–905. [CrossRef] [PubMed]

37. Spassova, M.A.; Soboloff, J.; He, L.P.; Xu, W.; Dziadek, M.A.; Gill, D.L. STIM1 has a plasma membrane role in the activation of store-operated Ca\textsuperscript{2+} channels. Proc. Natl. Acad. Sci. USA 2006, 103, 4040–4045. [CrossRef] [PubMed]

38. Mercer, J.C.; Dehaven, W.I.; Smyth, J.T.; Wedel, B.; Boyles, R.R.; Bird, G.S.; Putney, J.W. Large store-operated calcium selective currents due to co-expression of Orai1 or Orai2 with the intracellular calcium sensor, STIM1. J. Biol. Chem. 2006, 281, 24979–24990. [CrossRef] [PubMed]

39. Wu, M.M.; Buchanen, J.; Luik, R.M.; Lewis, R.S. Ca\textsuperscript{2+} store depletion causes STIM1 to accumulate in ER regions closely associated with the plasma membrane. J. Cell Biol. 2006, 174, 803–813. [CrossRef] [PubMed]

40. Brandman, O.; Liou, J.; Park, W.S.; Meyer, T. STIM2 is a feedback regulator that stabilizes basal cytosolic and endoplasmic reticulum Ca\textsuperscript{2+} levels. Cell 2007, 131, 1327–1339. [CrossRef] [PubMed]

41. Manji, S.S.; Parker, N.J.; Williams, R.T.; van Stekelenburg, L.; Pearson, R.B.; Dziadek, M.; Smith, P.J. STIM1: A novel phosphoprotein located at the cell surface. Biochim. Biophys. Acta 2000, 1481, 147–155. [CrossRef]

42. Shim, A.H.; Tirado-Lee, L.; Prakriya, M. Structural and functional mechanisms of CRAC channel regulation. J. Mol. Biol. 2015, 427, 77–93. [CrossRef] [PubMed]

43. Stathopulos, P.B.; Li, G.Y.; Plevin, M.J.; Ames, J.B.; Ikura, M. Stored Ca\textsuperscript{2+} depletion-induced oligomerization of stromal interaction molecule 1 (STIM1) via the EF-SAM region: An initiation mechanism for capacitative Ca\textsuperscript{2+} entry. J. Biol. Chem. 2006, 281, 35855–35862. [CrossRef] [PubMed]

44. Soboloff, J.; Spassova, M.A.; Hewavitharana, T.; He, L.P.; Xu, W.; Johnstone, L.S.; Dziadek, M.A.; Gill, D.L. STIM2 is an inhibitor of STIM1-mediated store-operated Ca\textsuperscript{2+} entry. Curr. Biol. 2006, 16, 1465–1470. [CrossRef] [PubMed]

45. Williams, R.T.; Manji, S.S.; Parker, N.J.; Hancock, M.S.; van Stekelenburg, L.; Eid, J.P.; Senior, P.V.; Kazenwadel, J.S.; Shandala, T.; Saint, R.; et al. Identification and characterization of the STIM (stromal interaction molecule) gene family: Coding for a novel class of transmembrane proteins. Biochem. J. 2001, 357, 673–685. [CrossRef]

46. Zheng, L.; Stathopulos, P.B.; Li, G.Y.; Ikura, M. Biophysical characterization of the EF-hand and SAM domain containing Ca\textsuperscript{2+} sensory region of STIM1 and STIM2. Biochem. Biophys. Res. Commun. 2008, 369, 240–246. [CrossRef] [PubMed]

47. Darbellay, B.; Arnaudeau, S.; Bader, C.R.; Konig, S.; Bernheim, L. STIM1 is a new actin-binding splice variant involved in fast repetitive Ca\textsuperscript{2+} release. J. Cell Biol. 2011, 194, 335–346. [CrossRef] [PubMed]

48. Miederer, A.M.; Alansary, D.; Schwär, G.; Lee, P.H.; Jung, M.; Helms, V.; Niemeyer, B.A. A STIM2 splice variant negatively regulates store-operated calcium entry. Nat. Commun. 2015, 6, 6899. [CrossRef] [PubMed]

49. Rana, A.; Yen, M.; Sadaghiani, A.M.; Malmersjö, S.; Park, C.Y.; Dolmetsch, R.E.; Lewis, R.S. Alternative splicing converts STIM2 from an activator to an inhibitor of store-operated calcium channels. J. Cell Biol. 2015, 209, 653–669. [CrossRef] [PubMed]

50. Hoth, M.; Niemeyer, B.A. The neglected CRAC proteins: Orai2, Orai3, and STIM2. Curr. Top. Membr. 2013, 71, 237–271. [PubMed]

51. Frischauf, I.; Schindl, R.; Bergsmann, J.; Derler, I.; Fahrner, M.; Muiik, M.; Fritsch, R.; Lackner, B.; Groschner, K.; Romani, C. Cooperativeness of Orai cytosolic domains tunes subtype-specific gating. J. Biol. Chem. 2011, 286, 8577–8584. [CrossRef] [PubMed]

52. Takahashi, Y.; Murakami, M.; Watanabe, H.; Hasegawa, H.; Ohba, T.; Munehisa, Y.; Nobori, K.; Ono, K.; Iijima, T.; Ito, H. Essential role of the N-terminus of murine Orai1 in store-operated Ca\textsuperscript{2+} entry. Biochem. Biophys. Res. Commun. 2007, 356, 45–52. [CrossRef] [PubMed]

53. Cahalan, M.D.; Zhang, S.L.; Yeromin, A.V.; Ohlsen, K.; Roos, J.; Stauderman, K.A. Molecular basis of the CRAC channel. Cell Calcium 2007, 42, 133–144. [CrossRef] [PubMed]

54. Frischauf, I.; Muiik, M.; Derler, I.; Bergsmann, J.; Fahrner, M.; Schindl, R.; Groschner, K.; Romani, C. Molecular determinants of the coupling between STIM1 and Orai channels: Differential activation of Orai1–3 channels by a STIM1 coiled-coil mutant. J. Biol. Chem. 2009, 284, 21696–21706. [CrossRef] [PubMed]
55. McNally, B.A.; Prakriya, M. Permeation, selectivity and gating in store-operated CRAC channels. J. Physiol. 2012, 590, 4179–4191. [CrossRef] [PubMed]

56. Derler, I.; Jardin, I.; Romanin, C. Molecular mechanisms of STIM/Orai communication. Circ. Res. 2008, 103, 1289–1299. [CrossRef] [PubMed]

57. Zhang, W.; Halligan, K.E.; Zhang, X.; Bisaillon, J.M.; Gonzalez-Cobos, J.C.; Motiani, R.K.; Hu, G.; Vincent, P.A.; Zhou, J.; Barroso, M.; et al. Orai1-mediated IC_{CRAC} is essential for neointima formation after vascular injury. Circ. Res. 2011, 109, 534–542. [CrossRef] [PubMed]

58. Gudlur, A.; Quintana, A.; Zhou, Y.; Hirve, N.; Mahapatra, S.; Hogan, P.G. STIM1 triggers a gating rearrangement at the extracellular mouth of the Orai1 channel. Nat. Commun. 2014, 5, 5164. [CrossRef] [PubMed]

59. Derler, I.; Jardin, I.; Romanin, C. Molecular mechanisms of STIM/Orai communication. Am. J. Physiol. Cell Physiol. 2016, 310, C643–C662. [PubMed]

60. Parekh, A.B. Store-operated CRAC channels: Function in health and disease. Nat. Rev. Drug Discov. 2010, 9, 399–410. [CrossRef] [PubMed]

61. Feske, S. Orai1 and STIM1 deficiency in human and mice: Roles of store-operated Ca^{2+} entry in the immune system and beyond. Immuno. Rev. 2009, 231, 189–209. [CrossRef] [PubMed]

62. Abdullaev, I.F.; Bisaillon, J.M.; Potier, M.; Gonzalez, J.C.; Motiani, R.K.; Trebak, M. STIM1 and Orai1 mediate CRAC currents and store-operated calcium entry important for endothelial cell proliferation. Circ. Res. 2008, 105, 1305–1313. [CrossRef] [PubMed]

63. Zhang, W.; Halligan, K.E.; Zhang, X.; Bisaillon, J.M.; Gonzalez-Cobos, J.C.; Motiani, R.K.; Hu, G.; Vincent, P.A.; Zhou, J.; Barroso, M.; et al. Orai1-mediated IC_{CRAC} is essential for neointima formation after vascular injury. Circ. Res. 2011, 109, 534–542. [CrossRef] [PubMed]

64. Stanisz, H.; Stark, A.; Kilch, T.; Schwarz, E.C.; Müller, C.S.; Peinelt, C.; Hoth, M.; Niemeyer, B.A.; Vogt, T.; Bogeski, I. Orai1 Ca^{2+} channels control endothelin-1-induced mitogenesis and melanogenesis in primary human melanocytes. J. Invest. Dermatol. 2012, 132, 1443–1451. [CrossRef] [PubMed]

65. Ohana, L.; Newell, E.W.; Stanley, E.F.; Schlichter, L.C. The Ca^{2+} release-activated Ca^{2+} current IC_{CRAC} mediates store-operated Ca^{2+} entry in rat microglia. Channels 2009, 3, 129–139. [CrossRef] [PubMed]

66. Barritt, G.J.; Litjens, T.L.; Castro, J.; Aromataris, E.; Rychkov, G.Y. Store-operated Ca^{2+} channels and microdomains of Ca^{2+} in liver cells. Clin. Exp. Pharmacol. Physiol. 2009, 36, 77–83. [CrossRef] [PubMed]

67. Stathopulos, P.B.; Zheng, L.; Ikura, M. Stromal interaction molecule (STIM) 1 and STIM2 calcium sensing regions exhibit distinct unfolding and oligomerization kinetics. J. Biol. Chem. 2009, 284, 728–732. [CrossRef] [PubMed]

68. Kar, P.; Bakowski, D.; di Capite, J.; Nelson, C.; Parekh, A.B. Different agonists recruit different stromal interaction molecule proteins to support cytoplasmic Ca^{2+} oscillations and gene expression. Proc. Natl. Acad. Sci. USA 2012, 109, 6969–6974. [CrossRef] [PubMed]

69. Hoover, P.J.; Lewis, R.S. Stoichiometric requirements for trapping and gating of Ca^{2+} release-activated Ca^{2+} (CRAC) channels by stromal interaction molecule 1 (STIM1). Proc. Natl. Acad. Sci. USA 2011, 108, 13299–13304. [CrossRef] [PubMed]

70. Li, Z.; Liu, L.; Deng, Y.; Ji, W.; Du, W.; Xu, P.; Chen, L.; Xu, T. Graded activation of CRAC channel by binding of different numbers of STIM1 to Orai1 subunits. Cell Res. 2011, 21, 305–315. [CrossRef] [PubMed]

71. Stathopulos, P.B.; Schindl, R.; Fahrner, M.; Zheng, L.; Gasm-Siebrook, G.M.; Muik, M.; Romanin, C.; Ikura, M. STIM1/Orai1 coiled-coil interplay in the regulation of store-operated calcium entry. Nat. Commun. 2013, 4, 2963. [CrossRef] [PubMed]

72. Venkatachalam, K.; Montell, C. TRP channels. Annu. Rev. Biochem. 2007, 76, 387–417. [CrossRef] [PubMed]

73. Birnbaumer, L. The TRPC class of ion channels: A critical review of their roles in slow, sustained increases in intracellular Ca^{2+} concentrations. Annu. Rev. Pharmacol. Toxicol. 2009, 49, 395–426. [CrossRef] [PubMed]

74. Lockwich, T.P.; Liu, X.; Singh, B.B.; Jadlowiec, J.; Weiland, S.; Ambudkar, I.S. Assembly of TRP1 in a signaling complex associated with caveolin-scaffolding lipid raft domains. J. Biol. Chem. 2000, 275, 11934–11942. [CrossRef] [PubMed]

75. Lockwich, T.; Singh, B.B.; Liu, X.; Ambudkar, I.S. Stabilization of cortical actin induces internalization of transient receptor potential 3 (TRP3)-associated caveolar Ca^{2+} signaling complex and loss of Ca^{2+} influx without disruption of TRP3-inositol trisphosphate receptor association. J. Biol. Chem. 2001, 276, 42401–42408. [CrossRef] [PubMed]
76. Vannier, B.; Zhu, X.; Brown, D.; Birnbaumer, L. The membrane topology of human transient receptor potential 3 as inferred from glycosylation-scanning mutagenesis and epitope immunocytochemistry. *J. Biol. Chem.* 1998, 273, 8675–8679. [CrossRef] [PubMed]

77. Strübing, C.; Krapivinsky, G.; Krapivinsky, L.; Clapham, D.E. Formation of novel TRPC channels by complex subunit interactions in embryonic brain. *J. Biol. Chem.* 2003, 278, 39014–39019. [CrossRef] [PubMed]

78. Hofmann, T.; Schaefer, M.; Schultz, G.; Güdermann, T. Subunit composition of mammalian transient receptor potential channels in living cells. *Proc. Natl. Acad. Sci. USA* 2002, 99, 7461–7466. [CrossRef] [PubMed]

79. Wedel, B.J.; Vazquez, G.; McKay, R.R.; Bird, G.S.J.; Putney, J.W. A calmodulin/inositol 1,4,5-trisphosphate (IP3) receptor-binding region targets TRPC3 to the plasma membrane in a calmodulin/IP3 receptor-independent process. *J. Biol. Chem.* 2003, 278, 25758–25765. [CrossRef] [PubMed]

80. Engelke, M.; Friedrich, O.; Budde, P.; Schäfer, C.; Niemann, U.; Zitt, C.; Jüngling, E.; Rocks, O.; Lückhoff, A.; Hofmann, T.; Schaefer, M.; Schultz, G.; Gudermann, T. Subunit composition of mammalian transient receptor potential channels. *FEBS Lett.* 2002, 523, 193–199. [CrossRef] [PubMed]

81. Singh, B.B.; Liu, X.; Tang, J.; Zhu, M.X.; Ambudkar, I.S. Calmodulin regulates Ca²⁺-dependent feedback inhibition of store-operated Ca²⁺ influx by interaction with a site in the C terminus of TRPC1. *Mol. Cell* 2002, 9, 739–750. [CrossRef]

82. Brazer, S.C.; Singh, B.B.; Liu, X.; Swaim, W.; Ambudkar, I.S. Caveolin-1 contributes to assembly of store-operated Ca²⁺ influx channels in living cells. Role of plasma membrane localization of TRPC1. *J. Biol. Chem.* 2003, 278, 27208–27215. [CrossRef] [PubMed]

83. Dohke, Y.; Oh, Y.S.; Ambudkar, I.S.; Turner, R.J. Biogenesis and topology of the transient receptor potential Ca²⁺ channel TRPC1. *J. Biol. Chem.* 2004, 279, 12242–12248. [CrossRef] [PubMed]

84. Kwan, H.Y.; Huang, Y.; Yao, X. Regulation of canonical transient receptor potential isoform 3 (TRPC3) channel by protein kinase G. *Proc. Natl. Acad. Sci. USA* 2004, 101, 2625–2630. [CrossRef] [PubMed]

85. Trebak, M.; Hempel, N.; Wedel, B.J.; Smyth, J.T.; Bird, G.S.; Putney, J.W. Negative regulation of TRPC3 channels by protein kinase C-mediated phosphorylation of serine 712. *Mol. Pharmacol.* 2005, 67, 558–563. [CrossRef] [PubMed]

86. Goel, M.; Sinkins, W.G.; Schilling, W.P. Selective association of TRPC channel subunits in rat brain synaptosomes. *J. Biol. Chem.* 2002, 277, 48303–48310. [CrossRef] [PubMed]

87. Ma, X.; Cheng, K.T.; Wong, C.O.; O’Neil, R.G.; Birnbaumer, L.; Ambudkar, I.S.; Yao, X. Heteromeric TRPV4-c1 channels contribute to store-operated Ca²⁺ entry in vascular endothelial cells. *Cell Calcium* 2011, 50, 502–509. [CrossRef] [PubMed]

88. Schindl, R.; Fritsch, R.; Jardin, I.; Frischauf, I.; Kahr, H.; Muiik, M.; Riedl, M.C.; Groschner, K.; Romanin, C. Canonical transient receptor potential (TRPC) 1 acts as a negative regulator for vanilloid TRPV6-mediated Ca²⁺ influx. *J. Biol. Chem.* 2012, 287, 35612–35620. [CrossRef] [PubMed]

89. Albarrán, L.; Lopez, J.J.; Dionisio, N.; Smani, T.; Salido, G.M.; Rosado, J.A. Transient receptor potential ankyrin-1 (TRPA1) modulates store-operated Ca²⁺ entry by regulation of STIM1-Orai1 association. *Biochim. Biophys. Acta* 2013, 1833, 3025–3034. [CrossRef] [PubMed]

90. Cheng, K.T.; Ong, H.L.; Liu, X.; Ambudkar, I.S. Contribution and regulation of TRPC channels in store-operated Ca²⁺ entry. *Curr. Top. Membr.* 2013, 71, 149–179. [PubMed]

91. Ong, H.L.; de Souza, L.B.; Ambudkar, I.S. Role of TRPC channels in store-operated calcium entry. *Adv. Exp. Med. Biol.* 2016, 898, 87–109. [PubMed]

92. Liu, X.; Singh, B.B.; Ambudkar, I.S. TRPC1 is required for functional store-operated Ca²⁺ channels. Role of acidic amino acid residues in the S5–S6 region. *J. Biol. Chem.* 2003, 278, 11337–11343. [CrossRef] [PubMed]

93. Liu, X.; Cheng, K.T.; Bandyopadhyay, B.C.; Pani, B.; Dietrich, A.; Paria, B.C.; Swaim, W.D.; Beech, D.; Yildirim, E.; Singh, B.B.; et al. Attenuation of store-operated Ca²⁺ current impairs salivary gland fluid secretion in TRPC1–/− mice. *Proc. Natl. Acad. Sci. USA* 2007, 104, 17542–17547. [CrossRef] [PubMed]

94. Lodola, F.; Laforenza, U.; Bonetti, E.; Lim, D.; Dragoni, S.; Bottino, C.; Ong, H.L.; Guerra, G.; Ganini, C.; Massa, M.; et al. Store-operated Ca²⁺ entry is remodelled and controls in vitro angiogenesis in endothelial progenitor cells isolated from tumoral patients. *PLoS ONE* 2012, 7, e42541. [CrossRef] [PubMed]

95. Fatherazi, S.; Presland, R.B.; Belton, C.M.; Goodwin, P.; Al-Qutub, M.; Trbic, Z.; Macdonald, G.; Schubert, M.M.; Iizutsu, K.T. Evidence that TRPC4 supports the calcium selective I_{CRAC},like current in human gingival keratinocytes. *Pflugers Arch.* 2007, 453, 879–889. [CrossRef] [PubMed]
96. Freichel, M.; Suh, S.H.; Pfeifer, A.; Schweig, U.; Trost, C.; Weissgerber, P.; Biel, M.; Philipp, S.; Freise, D.; Droogmans, G.; et al. Lack of an endothelial store-operated Ca\(^{2+}\) current impairs agonist-dependent vasorelaxation in TRP4\(^{-/-}\) mice. Nat. Cell Biol. 2001, 3, 121–127. [CrossRef] [PubMed]

97. Yang, H.; Mergler, S.; Sun, X.; Wang, Z.; Lu, L.; Bonanno, J.A.; Pleyer, U.; Reinaç, P.S. TRPC4 knockout suppresses epidermal growth factor-induced store-operated channel activation and growth in human corneal epithelial cells. J. Biol. Chem. 2005, 280, 32230–32237. [CrossRef] [PubMed]

98. Vázquez, G.; Wedel, B.J.; Trebak, M.; St John Bird, G.; Putney, J.W. Expression level of the canonical transient receptor potential 3 (TRPC3) channel determines its mechanism of activation. J. Biol. Chem. 2003, 278, 21649–21654. [CrossRef] [PubMed]

99. Ong, H.L.; de Souza, L.B.; Cheng, K.T.; Ambudkar, I.S. Physiological functions and regulation of TRPC channels. Handb. Exp. Pharmacol. 2014, 223, 1005–1034. [PubMed]

100. Zeng, W.; Yuan, J.P.; Kim, M.S.; Choi, Y.J.; Huang, G.N.; Worley, P.F.; Mullalem, S. STIM1 gates TRPC channels, but not Orai1, by electrostatic interaction. Mol. Cell 2008, 32, 439–448. [CrossRef] [PubMed]

101. Sundivakkam, P.C.; Freichel, M.; Singh, V.; Yuan, J.P.; Vogel, S.M.; Flockerzi, V.; Malik, A.B.; Tiruppathi, C.; Sundivakkam, P.C.; Freichel, M.; Suh, S.H.; Pfeifer, A.; Schweig, U.; Trost, C.; Weissgerber, P.; Biel, M.; Philipp, S.; Freise, D.; Freichel, M.; Suh, S.H.; Pfeifer, A.; Schweig, U.; Trost, C.; Weissgerber, P.; Biel, M.; Philipp, S.; Freise, D.; Italiano, J.E.; Lecine, P.; Shivdasani, R.A.; Hartwig, J.H. Blood platelets are assembled principally at the ends of proplatelet processes produced by differentiated megakaryocytes. J. Cell Biol. 1999, 147, 1299–1312. [CrossRef] [PubMed]
Nishimura, S.; Nagasaki, M.; Kunishima, S.; Sawaguchi, A.; Sakata, A.; Sakaguchi, H.; Ohmori, T.; Manabe, I.;
Di Buduo, C.A.; Currao, M.; Pecci, A.; Kaplan, D.L.; Balduini, C.L.; Balduini, A. Revealing eltrombopag’s
Italiano, J.E.; Shivdasani, R.A. Megakaryocytes and beyond: The birth of platelets. J. Thromb. Haemost. 2003,
Blair, P.; Flamenhaft, R. Platelet α-granules: Basic biology and clinical correlates. Blood Rev. 2009, 23,
Fava, R.A.; Casey, T.T.; Wilcox, J.; Pelton, R.W.; Moses, H.L.; Nanney, L.B. Synthesis of transforming growth
factor-β 1 by megakaryocytes and its localization to megakaryocyte and platelet α-granules. Blood 1990, 76,
Hamada, T.; Möhle, R.; Hesselgesser, J.; Hoxie, J.; Nachman, R.L.; Moore, M.A.; Rafii, S. Transendothelial
migration of megakaryocytes in response to stromal cell-derived factor 1 (SDF-1) enhances platelet formation.
J. Exp. Med. 1998, 188, 539–548. [CrossRef] [PubMed]
Avecilla, S.T.; Hattori, K.; Heissig, B.; Tejada, R.; Liao, F.; Shido, K.; Jin, D.K.; Dias, S.; Zhang, F.; Hartman, T.E.;
et al. Chemokine-mediated interaction of hematopoietic progenitors with the bone marrow vascular niche is
required for thrombopoiesis. Nat. Med. 2004, 10, 64–71. [CrossRef] [PubMed]
Junt, T.; Schulze, H.; Chen, Z.; Massberg, S.; Goerge, T.; Krueger, A.; Wagner, D.D.; Graf, T.; Italiano, J.E.;
Shivdasani, R.A.; et al. Dynamic visualization of thrombopoiesis within bone marrow. Science 2007, 317,
Zhang, L.; Orban, M.; Lorenz, M.; Barocke, V.; Braun, D.; Urtz, N.; Schulz, C.; von Brühl, M.L.; Tirmiceriu, A.;
Gaertner, F.; et al. A novel role of sphingosine 1-phosphate receptor S1pr1 in mouse thrombopoiesis.
J. Exp. Med. 2012, 209, 2165–2181. [CrossRef] [PubMed]
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]
Balduini, A.; di Buduo, C.A.; Kaplan, D.L. Translational approaches to functional platelet production ex vivo.
Thromb. Haemost. 2016, 115, 250–256. [CrossRef] [PubMed]
Sim, X.; Poncz, M.; Gadue, P.; French, D.L. Understanding platelet generation from megakaryocytes:
Implications for in vitro-derived platelets. Blood 2016, 127, 1227–1233. [CrossRef] [PubMed]
Di Buduo, C.A.; Currao, M.; Pecci, A.; Kaplan, D.L.; Balduini, C.L.; Balduini, A. Revealing eltrombopag’s
promotion of human megakaryopoiesis through AKT/ERK-dependent pathway activation. Haematologica
2016, 125, 2254–2264.
Long, M.W. Megakaryocyte differentiation events. Semin. Hematol. 1998, 35, 192–199. [PubMed]
Nishimura, S.; Nagasaki, M.; Kunishima, S.; Sawaguchi, A.; Sakata, A.; Sakaguchi, H.; Ohmori, T.; Manabe, I.;
Italiano, J.E.; Ryu, T.; et al. II-1α induces thrombopoiesis through megakaryocyte rupture in response to
acute platelet needs. J. Exp. Med. 2015, 209, 453–466. [CrossRef] [PubMed]
Lo Celso, C.; Fleming, H.E.; Wu, J.W.; Zhao, C.X.; Mlake-Lye, S.; Fujisaki, J.; Côté, D.; Rowe, D.W.; Lin, C.P.;
Scadden, D.T. Live-animal tracking of individual hematopoietic stem/progenitor cells in their niche. Nature
2009, 457, 92–96. [CrossRef] [PubMed]
Arai, F.; Hiroa, A.; Ohmura, M.; Sato, H.; Matsuoka, S.; Takubo, K.; Ito, K.; Koh, G.Y.; Suda, T. Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche.
Cell 2004, 118, 149–161. [CrossRef] [PubMed]
Fliedner, T.M.; Calvo, W.; Klinnert, V.; Nothdurft, W.; Prüimmer, O.; Raghavachar, A. Bone marrow structure
and its possible significance for hematopoietic cell renewal. Ann. N. Y. Acad. Sci. 1985, 459, 73–84. [CrossRef]
[PubMed]
Malar, A.; Gruppi, C.; Rebuzzini, P.; Visai, L.; Perotti, C.; Moratti, R.; Balduini, C.; Tira, M.E.; Balduini, A. Megakaryocyte-matrix interaction within bone marrow: New roles for fibronectin and factor XIII-A. Blood
2011, 117, 2476–2483. [CrossRef] [PubMed]
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] [PubMed]
Balduini, A.; Pallotta, I.; Malar, 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]
137. Malara, A.; Currao, M.; Gruppi, C.; Celesti, G.; Viareno, 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]

138. Abbonante, V.; Gruppi, C.; Catarsi, P.; Avanzini, M.A.; Tira, M.E.; Barosi, G.; Rosti, V.; Balduini, A. Altered fibronectin expression and deposition by myeloproliferative neoplasm-derived mesenchymal stromal cells. *Br. J. Haematol.* 2015, 172, 140–144. [CrossRef] [PubMed]

139. Kuter, D.J.; Bain, B.; Mufti, G.; Bagg, A.; Hasserjian, R.P. Bone marrow fibrosis: Pathophysiology and clinical significance of increased bone marrow stromal fibres. *Br. J. Haematol.* 2007, 139, 351–362. [CrossRef] [PubMed]

140. Kimura, A.; Katoh, O.; Hyodo, H.; Kuramoto, A. Transforming growth factor-$\beta$ regulates growth as well as collagen and fibronectin synthesis of human marrow fibroblasts. *Br. J. Haematol.* 1989, 72, 486–491. [CrossRef] [PubMed]

141. Abbonante, V.; di Buduo, C.A.; Gruppi, C.; Malara, A.; Gianelli, U.; Celesti, G.; Anselmo, A.; Laghi, L.; Vercellino, M.; Visai, L.; et al. Thrombopoietin/TGF-$\beta_1$ loop regulates megakaryocyte extracellular matrix component synthesis. *Stem Cells* 2016, 34, 1123–1133. [CrossRef] [PubMed]

142. Badalucco, S.; di Buduo, C.A.; Campanelli, R.; Pallotta, I.; Catarsi, P.; Rosti, V.; Kaplan, D.L.; Barosi, G.; Massa, M.; Balduini, A. Involvement of TGF$\beta_1$ in autocrine regulation of proplatelet formation in healthy subjects and patients with primary myelofibrosis. *Haematologica* 2013, 98, 514–517. [CrossRef] [PubMed]

143. Balduini, A.; di Buduo, C.A.; Malara, A.; Lecchi, A.; Rebuzzini, P.; Currao, M.; Pallotta, I.; Jakubowski, J.A.; Cattaneo, M. Constitutively released adenosine diphosphate regulates proplatelet formation by human megakaryocytes. *Haematologica* 2012, 97, 1657–1665. [CrossRef] [PubMed]

144. Casella, I.; Feccia, T.; Chelucci, C.; Samoggia, P.; Castelli, G.; Guerrieri, R.; Parolini, I.; Petrucci, E.; Pelosi, E.; Morsilli, O.; et al. Autocrine-paracrine VEGF loops potentiate the maturation of megakaryocytic precursors through Flt1 receptor. *Blood* 2003, 101, 1316–1323. [CrossRef] [PubMed]

145. Möhle, R.; Green, D.; Moore, M.A.; Nachman, R.L.; Rafii, S. Constitutive production and thrombin-induced release of vascular endothelial growth factor by human megakaryocytes and platelets. *Proc. Natl. Acad. Sci. USA* 1997, 94, 663–668. [CrossRef] [PubMed]

146. Lambert, M.P.; Rauova, L.; Bailey, M.; Sola-Visner, M.C.; Kowalska, M.A.; Poncz, M. Platelet factor 4 is a negative autocrine in vivo regulator of megakaryopoiesis: Clinical and therapeutic implications. *Blood* 2007, 110, 1153–1160. [CrossRef] [PubMed]

147. Malara, A.; Abbonante, V.; di Buduo, C.A.; Tozzi, L.; Currao, M.; Balduini, A. The secret life of a megakaryocyte: Emerging roles in bone marrow homeostasis control. *Cell. Mol. Life Sci.* 2015, 72, 1517–1536. [CrossRef] [PubMed]

148. Di Buduo, C.A.; Alberelli, M.A.; Glembostky, A.C.; Podda, G.; Lev, P.R.; Cattaneo, M.; Landolfi, R.; Heller, P.G.; Balduini, A.; de Candia, E. Abnormal proplatelet formation and emperipolesis in cultured human megakaryocytes from gray platelet syndrome patients. *Sci. Rep.* 2016, 6, 23213. [CrossRef] [PubMed]

149. Balduini, C.L.; Pecci, A.; Noris, P. Diagnosis and management of inherited thrombocytopenias. *Semin. Thromb. Hemost.* 2013, 39, 161–171. [PubMed]

150. Eto, K.; Kunishima, S. Linkage between the mechanisms of thrombocytopenia and thrombopoiesis. *Blood* 2016, 127, 1234–1241. [CrossRef] [PubMed]

151. Williams, N.; Levine, R.F. The origin, development and regulation of megakaryocytes. *Br. J. Haematol.* 1982, 52, 173–180. [CrossRef] [PubMed]

152. Ru, Y.X.; Zhao, S.X.; Dong, S.X.; Yang, Y.Q.; Eydén, B. On the maturation of megakaryocytes: A review with original observations on human in vivo cells emphasizing morphology and ultrastructure. *Ultrastuct. Pathol.* 2015, 39, 79–87. [CrossRef] [PubMed]

153. Eckly, A.; Heijnen, H.; Pertuy, F.; Geerts, W.; Proux, F.; Rinckel, J.Y.; Léon, C.; Lanza, F.; Gachet, C. Biogenesis of the demarcation membrane system (DMS) in megakaryocytes. *Blood* 2014, 123, 921–930. [CrossRef] [PubMed]

154. Lacabaratz-Porret, C.; Lanay, S.; Corvazier, E.; Bredoux, R.; Papp, B.; Enouf, J. Biogenesis of endoplasmic reticulum proteins involved in Ca$^{2+}$ signalling during megakaryocytic differentiation: An in vitro study. *Biochem. J.* 2000, 350, 723–734. [CrossRef] [PubMed]
Sun, S.; Liu, Y.; Lipsky, S.; Cho, M. Physical manipulation of calcium oscillations facilitates
Putney, J.W.; Bird, G.S. Cytoplasmic calcium oscillations and store-operated calcium influx.
Wakabayashi, I.; Marumo, M.; Graziani, A.; Poteser, M.; Groschner, K. TRPC4 expression determines
Nurden, P.; Debili, N.; Vainchenker, W.; Bobe, R.; Bredoux, R.; Corvazier, E.; Comrie, R.; Fressinaud, E.; Meyer, D.; Nurden, A.T.; et al. Impaired megakaryocytopoiesis in type 2B von Willebrand disease with severe thrombocytopenia. *Blood* **2006**, *108*, 2587–2595. [CrossRef] [PubMed]
Sugiyama, T.; Yamamoto-Hino, M.; Miyawaki, A.; Furuichi, T.; Mikoshiba, K.; Hasegawa, M. Subtypes of inositol 1,4,5-triphosphate receptor in human hematopoietic cell lines: Dynamic aspects of their cell-type specific expression. *FEBS Lett.* **1994**, *349*, 191–196. [CrossRef]
Berg, L.P.; Shamsher, M.K.; El-Daher, S.S.; Kakkar, V.V.; Authi, K.S. Expression of human TRPC genes in the megakaryocytic cell lines MEG01, DAMI and HEL. *FEBS Lett.* **1997**, *403*, 83–86. [CrossRef]
Wakabayashi, I.; Marumo, M.; Graziani, A.; Poteser, M.; Groschner, K. TRPC4 expression determines sensitivity of the platelet-type capacitative Ca²⁺ entry channel to intracellular alkalosis. *Platelets* **2006**, *17*, 454–461. [CrossRef] [PubMed]
Den Dekker, E.; Molin, D.G.; Breikers, G.; van Oerle, R.; Akkerman, J.W.; van Eys, G.J.; Heemskerk, J.W. Expression of transient receptor potential mrna isoforms and Ca²⁺ influx in differentiating human stem cells and platelets. *Biochim. Biophys. Acta* **2001**, *1539*, 243–255. [CrossRef]
Den Dekker, E.; Gorter, G.; van der Vuurst, H.; Heemskerk, J.W.; Akkerman, J.W. Biogenesis of G-protein mediated calcium signaling in human megakaryocytes. *Thromb. Haemost.* **2001**, *86*, 1106–1113. [PubMed]
Putney, J.W.; Bird, G.S. Cytoplasmic calcium oscillations and store-operated calcium influx. *J. Physiol.* **2008**, *586*, 3055–3059. [CrossRef] [PubMed]
Wedel, B.; Boyles, R.R.; Putney, J.W.; Bird, G.S. Role of the store-operated calcium entry proteins STIM1 and Orai1 in muscarinic cholinergic receptor-stimulated calcium oscillations in human embryonic kidney cells. *J. Physiol.* **2007**, *579*, 679–689. [CrossRef] [PubMed]
Shuttleworth, T.J. What drives calcium entry during [Ca²⁺], oscillations?—Challenging the capacitative model. *Cell Calcium* **1999**, *25*, 237–246. [CrossRef] [PubMed]
Pinto, M.C.; Tonelli, F.M.; Vieira, A.L.; Kihara, A.H.; Ulrich, H.; Resende, R.R. Studying complex system: Calcium oscillations as attractor of cell differentiation. *Integr. Biol.* **2016**, *8*, 130–148. [CrossRef] [PubMed]
Sun, S.; Liu, Y.; Lipsky, S.; Cho, M. Physical manipulation of calcium oscillations facilitates oseodifferentiation of human mesenchymal stem cells. *FASEB J.* **2007**, *21*, 1472–1480. [CrossRef] [PubMed]
Parkash, J.; Asotra, K. Calcium wave signaling in cancer cells. *Life Sci.* **2010**, *87*, 587–595. [CrossRef] [PubMed]
Heise, N.; Palme, D.; Misovic, M.; Koka, S.; Rudner, J.; Lang, F.; Salih, H.R.; Huber, S.M.; Henke, G. Non-selective cation channel-mediated Ca²⁺-entry and activation of Ca²⁺/calmodulin-dependent kinase II contribute to G2/M cell cycle arrest and survival of irradiated leukemia cells. *Cell. Physiol. Biochem.* **2010**, *26*, 597–608. [CrossRef] [PubMed]
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]
Carter, R.N.; Tolhurst, G.; Walmsley, G.; Vizuete-Forster, M.; Miller, N.; Mahaut-Smith, M.P. Molecular and electrophysiological characterization of transient receptor potential ion channels in the primary murine megakaryocyte. *J. Physiol.* **2006**, *576*, 151–162. [CrossRef] [PubMed]
Varga-Szabo, D.; Authi, K.S.; Braun, A.; Bender, M.; Ambily, A.; Hassock, S.R.; Gudermann, T.; Dietrich, A.; Nieswandt, B. Store-operated Ca²⁺ entry in platelets occurs independently of transient receptor potential (TRP) C1. *Pflugers Arch.* **2008**, *457*, 377–387. [CrossRef] [PubMed]
Ramanathan, G.; Gupta, S.; Thielmann, I.; Pleines, L.; 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] [PubMed]
Borst, O.; Schmidt, E.M.; Münzer, P.; Schönberger, T.; Towhid, S.T.; Elvers, M.; Leibrock, C.; Schmid, E.; Eyleinstein, A.; Kuhl, D.; et al. The serum- and glucocorticoid-inducible kinase 1 (SGK1) influences platelet calcium signaling and function by regulation of Orai1 expression in megakaryocytes. *Blood* **2012**, *119*, 251–261. [CrossRef] [PubMed]
López, E.; Bena-Erro, A.; Salido, G.M.; Rosado, J.A.; Redondo, P.C. FKBP52 is involved in the regulation of SOCE channels in the human platelets and MEG 01 cells. *Biochim. Biophys. Acta* **2013**, *1833*, 652–662. [CrossRef] [PubMed]
174. Endo, T.; Kusakabe, M.; Sunadome, K.; Yamamoto, T.; Nishida, E. The kinase SGK1 in the endoderm and mesoderm promotes ectodermal survival by down-regulating components of the death-inducing signaling complex. Sci. Signal. 2011, 4, ra2. [CrossRef] [PubMed]

175. Lang, F.; Böhmer, C.; Palma, M.; Sebohm, G.; Strutz-Seebohm, N.; Vallon, V. (Patho) physiological significance of the serum- and glucocorticoid-inducible kinase isoforms. Physiol. Rev. 2006, 86, 1151–1178. [CrossRef] [PubMed]

176. Borst, O.; Münzer, P.; Schmid, E.; Schmidt, E.M.; Russo, A.; Walker, B.; Yang, W.; Leibrock, C.; Szteyn, K.; Schmidt, S.; et al. 1,25(OH)2 vitamin D3-dependent inhibition of platelet Ca2+ signaling and thrombus formation in klotho-deficient mice. FASEB J. 2014, 28, 2108–2119. [CrossRef] [PubMed]

177. Yan, J.; Schmid, E.; Almilaji, A.; Shumilina, E.; Borst, O.; Laufer, S.; Gawaz, M.; Lang, F. Effect of TGFβ on calcium signaling in megakaryocytes. Biochem. Biophys. Res. Commun. 2015, 461, 8–13. [CrossRef] [PubMed] [PubMed]

178. Almilaji, A.; Yan, J.; Hosseinizadeh, Z.; Schmid, E.; Gawaz, M.; Lang, F. Up-regulation of Na+/Ca2+ exchange in megakaryocytes following TGFβ1 treatment. Cell. Physiol. Biochem. 2016, 39, 693–699. [CrossRef] [PubMed]

179. Björquist, A.; di Buduo, C.A.; Femia, E.A.; Storey, R.F.; Becker, R.C.; Almilaji, A.; Nylander, S.; Cattaneo, M. Studies of the interaction of ticagrelor with the P2Y13 receptor and with P2Y13-dependent pro-platelet formation by human megakaryocytes. Thromb. Haemost. 2016, 116, 1079–1088. [CrossRef] [PubMed]

180. Mountford, J.C.; Melford, S.K.; Bunce, C.M.; Gibbins, J.; Watson, S.P. Collagen or collagen-related peptide cause [Ca2+]i elevation and increased tyrosine phosphorylation in human megakaryocytes. Thromb. Haemost. 1999, 82, 1153–1159. [PubMed]

181. Kamal, T.; Green, T.N.; Morel-Kopp, M.C.; Ward, C.M.; McGregor, A.L.; McGlashan, S.R.; Bohlander, S.K.; Browett, P.J.; Teague, L.; During, M.J.; et al. Inhibition of glutamate regulated calcium entry into leukemic megakaryoblasts reduces cell proliferation and supports differentiation. Cell Signal. 2015, 27, 1860–1872. [CrossRef] [PubMed]

182. Grosse, J.; Braun, A.; Varga-Szabo, D.; Beyersdorf, N.; Schneider, B.; Zeitlmann, L.; Hanke, P.; Schropp, P.; Mühlstedt, S.; Zorn, C.; et al. An EF hand mutation in STIM1 causes premature platelet activation and bleeding in mice. J. Clin. Investig. 2007, 117, 3540–3550. [CrossRef] [PubMed]

183. Tefferi, A. Myeloproliferative neoplasms: A decade of discoveries and treatment advances. Am. J. Hematol. 2016, 91, 50–58. [CrossRef] [PubMed]

184. Bianchi, E.; Norfo, R.; Pennucci, V.; Zini, R.; Manfredini, R. Genomic landscape of megakaryopoiesis and platelet function defects. Blood 2016, 127, 1249–1259. [CrossRef] [PubMed]

185. Thiele, J.; Kvasnicka, H.; Tefferi, A.; Barosi, G.; Orazi, A.; Vardiman, J. Primary myelofibrosis: Sverdlov, S.H., Campo, E., Harris, N.L., Jaffee, E.S., Pileri, S.A., Stein, H., Thiele, J., Vardiman, J.W., Eds.; IARC Press: Lyon, France, 2008; pp. 44–47.

186. Mountford, J.C.; Melford, S.K.; Bunce, C.M.; Gibbins, J.; Watson, S.P. Collagen or collagen-related peptide cause [Ca2+]i elevation and increased tyrosine phosphorylation in human megakaryocytes. Thromb. Haemost. 1999, 82, 1153–1159. [PubMed]

187. Camprubí, E.; Carpentier, V.C.; Menard, M.; Vérin, D.; Chachoua, I.; Pecquet, C.; El-Khoury, M.; Nivarthi, H.; Albu, R.I.; Marty, C.; Gryshkova, V.; Defour, J.P.; Vertenoël, G.; Ngo, A.; et al. Thrombopoietin receptor activation by myeloproliferative neoplasm associated calreticulin mutants. Blood 2016, 127, 1325–1335. [CrossRef] [PubMed]
192. Marty, C.; Pecquet, C.; Nivarthi, H.; El-Khoury, M.; Chachoua, I.; Tulliez, M.; Villeval, J.L.; Raslova, H.; Kralovics, R.; Constantinescu, S.N.; et al. Calreticulin mutants in mice induce an MPL-dependent thrombocytosis with frequent progression to myelofibrosis. Blood 2016, 127, 1317–1324. [CrossRef] [PubMed]

193. Michalak, M.; Corbett, E.F.; Mesaeli, N.; Nakamura, K.; Opas, M. Calreticulin: One protein, one gene, many functions. Biochem. J. 1999, 344, 281–292. [CrossRef] [PubMed]

194. Pietra, D.; Rumi, E.; Ferretti, V.V.; Buduo, C.A.; Milanesi, C.; Cavalloni, C.; Sant’Antonio, E.; Abbonante, V.; Moccia, F.; Casetti, I.C.; et al. Differential clinical effects of different mutation subtypes in CALR-mutant myeloproliferative neoplasms. Leukemia 2016, 30, 431–438. [CrossRef] [PubMed]

195. Bastianutto, C.; Clementi, E.; Codazzi, F.; Podini, P.; de Giorgi, F.; Rizzuto, R.; Meldolesi, J.; Pozzan, T. Overexpression of calreticulin increases the Ca\(^{2+}\) capacity of rapidly exchanging Ca\(^{2+}\) stores and reveals aspects of their luminal microenvironment and function. J. Cell Biol. 1995, 130, 847–855. [CrossRef] [PubMed]

196. Fasolato, C.; Pizzo, P.; Pozzan, T. Delayed activation of the store-operated calcium current induced by calreticulin overexpression in RBL-1 cells. Mol. Biol. Cell 1998, 9, 1513–1522. [CrossRef] [PubMed]

197. Xu, W.; Longo, F.J.; Wintermantel, M.R.; Jiang, X.; Clark, R.A.; DeLisle, S. Calreticulin modulates capacitative Ca\(^{2+}\) influx by controlling the extent of inositol 1,4,5-trisphosphate-induced Ca\(^{2+}\) store depletion. J. Biol. Chem. 2000, 275, 36676–36682. [CrossRef] [PubMed]

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