Mini-Review

Role of the store-operated Ca^{2+} channel in ATP-induced Ca^{2+} signalling in mesenchymal stem cells and regulation of cell functions

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1. Abstract

It is well-known that extracellular ATP acts as an autocrine/paracrine signal to regulate cell functions by inducing intracellular Ca^{2+} signalling through its cognate receptors, namely, the ligand-gated ion channel P2X receptors that mediate Ca^{2+} influx and/or the $G_{q/11}$-coupled P2Y receptors that link to Ca^{2+} release from the ER. The reduction in ER Ca^{2+} can trigger further extracellular Ca^{2+} entry by activating the store-operated Ca^{2+} (SOC) channel. Mesenchymal stem cells (MSC) play an important role in the homeostasis of residing tissues and have promising applications in regenerative medicines. MSC can release ATP spontaneously or in response to diverse stimuli, and express multiple P2X and $G_{q/11}$-coupled P2Y receptors that participate in ATP-induced Ca^{2+} signalling and regulate cell function. There is increasing evidence to show the contribution of the SOC channel in ATP-induced Ca^{2+} signalling in MSC. In this mini-review, we discuss the current understanding of the expression of the SOC channel in MSC and its potential role in mediating ATP-induced Ca^{2+} signalling and regulation of MSC differentiation, proliferation and migration.

2. Introduction

Adenosine 5'-triphosphate (ATP) is present intracellularly as the major energy source for a myriad of biochemical reactions and physiological processes that are critical to the viability and normal functions of cells in living organisms. Conceivably, ATP release into extracellular space occurs as a sequel or an indicator of tissue damage that causes cells to lose the integrity of the plasma membrane (PM), and ATP acts as a danger-associated molecular pattern molecule. There is also an extensive collection of evidence to show that many mammalian cell types can release ATP in a non-lytic fashion under physiological and pathological conditions [1–4]. It has been well established that ATP, once outside the cell, acts as an autocrine signal regulating multiple cell functions, or as a paracrine signal enabling cell-to-cell communication [5–7]. Intracellular Ca^{2+} is a ubiquitous second messenger that stimulates Ca^{2+}-dependent signal pathways underpinning the short-term and/or long-term effects of numerous external stimuli or signals on a wide range of cell functions [8]. Not surprisingly, the most common action modality of extracellular ATP as a signalling molecule

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is to raise intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) [9]. More specifically, ATP can generate intracellular Ca^{2+} signals with spatiotemporal dynamics through a family of cell surface receptors termed P2 purinergic receptors, which can be categorised into two functionally and structurally distinct subfamilies, P2X and P2Y [10]. Mammalian cells express seven P2X proteins or receptor subunits (P2X1–P2X7) [11], which have a membrane topology composed of intracellular N- and C-termini, two transmembrane domains and an exceptionally large extracellular domain, and can form homo/hetero-trimeric ATP-gated Ca^{2+}-permeable cation channels (Fig. 1A) [12–14]. There are eight different P2Y receptors in humans ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2Y_{11}$-$P2Y_{14}$), structurally all belonging to the seven-transmembrane domain guanosine diphosphate (GDP)/guanosine triphosphate (GTP)-binding protein (G protein)-coupled receptor superfamily. They display a differential sensitivity to extracellular ATP and various other nucleotides (e.g., UTP, UDP, ADP, and UDP-galactose) and coupling with different G-proteins and downstream signal pathways [15]. ATP preferentially activates the $P2Y_1$, $P2Y_2$ and $P2Y_{11}$ receptors, all of which are coupled to the $G_{q/11}$ protein, with the $P2Y_{11}$ receptor known to link alternatively with the G_s protein. Activation of these $G_{q/11}$-coupled receptors stimulates phospholipase C (PLC) to generate inositol 1,4,5-triphosphate (IP_3) from membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP_2), and IP_3 in turn opens the Ca^{2+}-release channel IP_3 receptor (IP_3R) in the endoplasmic reticulum (ER) (or sarcoplasmic reticulum in muscles), resulting in Ca^{2+} release from the ER (Fig. 1A). Constitutively, a majority of studies examining ATP-induced Ca^{2+} signaling and regulation of cell function have drawn attention to the P2X and P2Y receptors. However, it is widely documented that the reduction in the ER Ca^{2+} level can trigger the so-called store-operated Ca^{2+} entry (SOCE), through the store-operated Ca^{2+} (SOC) channel, to restore intracellular Ca^{2+} homeostasis, particularly the Ca^{2+} content in the ER [16, 17]. This Ca^{2+} entry mechanism, while initially and mostly reported in non-excitable cells, is also widely utilized in excitable cells [18, 19]. For example, a variety of neurotransmitters and neuromodulators, with ATP being one of them, activate their cognate $G_{q/11}$-coupled receptors to induce ER Ca^{2+} release and subsequent SOCE to shape neuronal Ca^{2+} signaling [18]. It is recognized nowadays that SOCE is one of the most common Ca^{2+} signalling mechanisms [16].

Mesenchymal stem cells (MSC) are present in stem cell niches in many adult tissues, like bone marrow, adipose tissue and dental pulp, and play an essential role in the homeostasis of residing tissues [20]. They are multipotent stem cells and able to differentiate into several cell lineages [21, 22]. Decades of studies have demonstrated their promising applications in regenerative medicines. MSC represent an attractive source of cells for tissue engineering to repair, regenerate or replace damaged or lost tissues (e.g., [23–34]). There are extensive interests in, and emerging evidence to support, the use of MSC in cell-based therapies to treat a variety of pathological conditions (e.g., [35–53]). A multiplicity of extracellular stimuli or signals, physical, chemical or biological, have been identified to regulate MSC functions and fate (e.g., [54–74]).

ATP represents one extracellular signal that regulates MSC differentiation, proliferation, migration and tissue homing [75–83]. It is well known that MSC exhibit a high sensitivity to diverse mechanical forces, for example, fluid flow-induced shear stress and shockwaves, as well as the mechanical properties of residing tissues and cell-supporting matrix. Such mechanical signals have been shown to significantly regulate MSC functions [77, 78, 84–92]. Interestingly, accumulating evidence from examining MSC and other mechanosensitive cells suggests that ATP release and induction of P2X/P2Y receptor-mediated Ca^{2+} signalling represent an important mechanism that transduces the mechanical signals into adaptative cell functions [93]. Multiple P2X and P2Y receptors are reported for their expression in MSC preparations from different species and tissues, albeit with some noticeable variations in the receptor type, expression level and role in ATP-induced Ca^{2+} signalling (reviewed by [94]). In addition to the P2X7 receptor, $P2Y_1$, $P2Y_2$ and $P2Y_{11}$ are the major receptors that participate in mediating ATP-induced Ca^{2+} signalling (Fig. 1A) [75–83, 95–97]. There is some evidence to show that SOCE can be induced in MSC by the reduction in ER Ca^{2+} following activation of the bespoke $G_{q/11}$-coupled P2Y receptors. However, it remains less well understood with respect to the contribution of SOCE in ATP-induced Ca^{2+} signalling in MSC. This min-review article aims to provide an overview of studies, particularly the recent studies from our own and also from other groups that evolve our understanding towards the molecular identity of the SOC channel in MSC and its role in ATP-induced Ca^{2+} signalling and, additionally, its potential role in ATP-induced regulation of cell differentiation, proliferation and migration.

3. The SOC/CRAC channel

As already introduced above, the SOC channel is activated by the loss of ER Ca^{2+} and thus, by its unique activation mode, is distinguished from the receptor-operated, ligand-gated or voltage-gated Ca^{2+} channels. Experimentally, SOC channel activation or SOCE can be readily induced by depleting the ER Ca^{2+} using thapsigargin (TG) to block the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) that mediates Ca^{2+} uptake from the cytosol into the ER (Fig. 1A), circumventing the need for activating the $G_{q/11}$-PLC-IP_3R pathway. Thus, as illustrated in Fig. 1B, one widely used means to demonstrate the SOCE is treating cells with TG in the absence of extracellular Ca^{2+} and measuring the $[Ca^{2+}]_i$ upon addition of extracellular Ca^{2+}, an experimental protocol often referred to as "Ca^{2+} add-back".

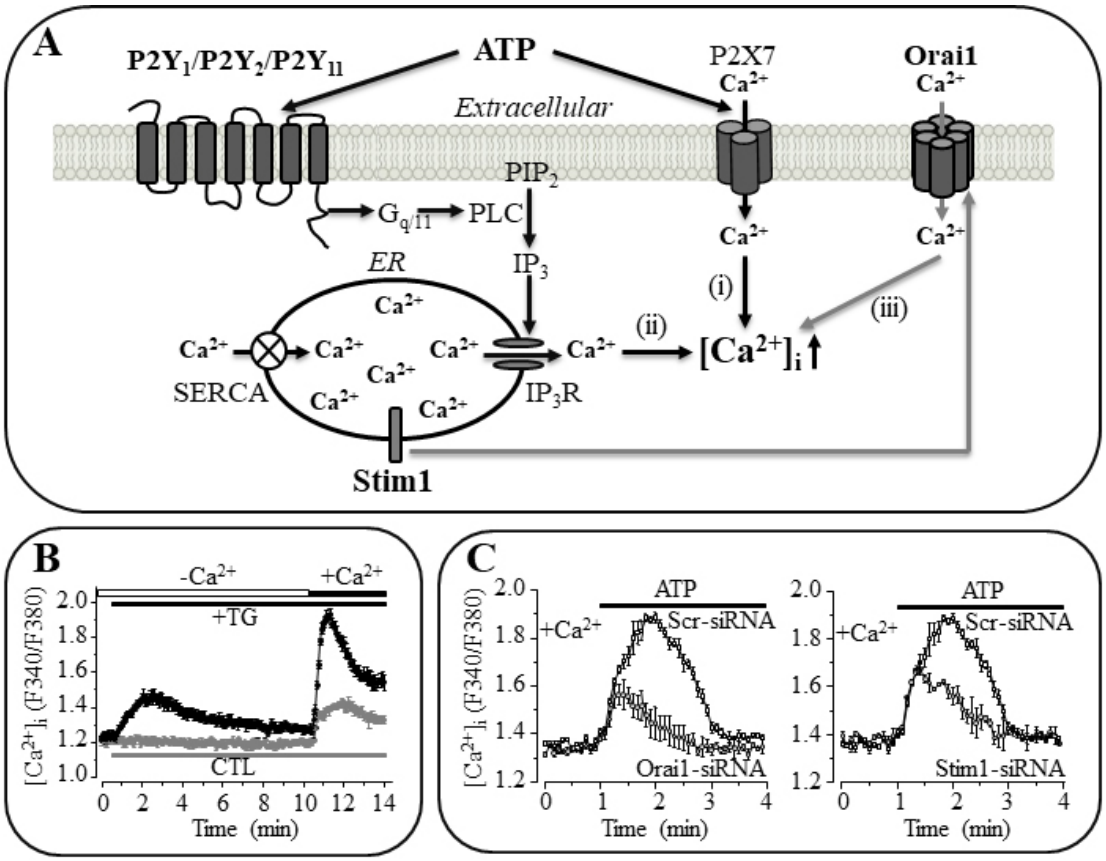

Fig. 1. A graphic illustration of the molecular mechanisms that participate in ATP-induced Ca^{2+} signalling in mesenchymal stem cells (MSC). (A) Extracellular ATP induces an increase in intracellular Ca^{2+} concentration via the P2X7 receptor that mediates Ca^{2+} influx (i). Alternatively, ATP activates the $G_{q/11}$-coupled P2Y receptor ($P2Y_1$, $P2Y_2$ and/or $P2Y_{11}$) and phospholipase C (PLC) to generate inositol 1,4,5-triphosphate (IP_3) from membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP_2) and induce IP_3 receptor (IP_3R)-mediated Ca^{2+} release from the endoplasmic reticulum (ER) (ii). Release of the ER Ca^{2+} subsequently triggers store-operated Ca^{2+} entry (SOCE) through the store-operated Ca^{2+} (SOC) channel, particularly the Ca^{2+}-release-activated Ca^{2+} (CRAC) channel (iii). Inhibition of the sarcoplasmic/endoplasmic Ca^{2+}-ATPase (SERCA) with thapsigargin (TG) to prevent the cytosolic Ca^{2+} uptake can lead to loss of the ER Ca^{2+}, which is widely used in "Ca^{2+} add-back" experiments to activate the SOC channel. (B) Example recordings using "Ca^{2+} add-back" to show that treatment of human dental pulp-derived MSC with TG induced release of the ER Ca^{2+} in the absence of extracellular Ca^{2+} led to a greater Ca^{2+} response upon re-introduction of extracellular Ca^{2+}. CTL, control (without TG treatment). (C) Example recordings showing that small interference RNA (siRNA)-mediated knockdown of the expression of Orai1 or Stim1 reduced ATP-induced Ca^{2+} response in human dental pulp MSC. Scr-siRNA, scrambled siRNA. (B) and (C) taken and modified from Peng *et al.* (2016) [80].

Early electrophysiological studies show that the Ca^{2+} permeability of the SOC channels varies considerably from being modest to highly selective, depending on the cells in which they are expressed [98]. So far, not all of the SOC channel-forming proteins have not been molecularly identified or established, and the Ca^{2+}-release-activated Ca^{2+} (CRAC) channel represents the best understood SOC channel. The CRAC channel displays several hallmark electrophysiological properties, including highly selective Ca^{2+} permeability (conducting Ca^{2+} >1000 times better than Na^+ under physiological conditions), tiny single channel unitary conductance (9–24 fS in 2–110 mM extracellular Ca^{2+}) and strong inward rectification [16]. Several candidates, including members of the transient receptor potential (TRP) channel superfamily, were proposed to form or function as the CRAC channel [16]. It is now mostly accepted that the families of Orai proteins and Ca^{2+}-sensing stromal interaction molecule (Stim) proteins, particularly Orai1 and Stim1, are the two core components of the CRAC channel [16, 99, 100]. These two proteins have distinctive structural features, subcellular location and role in the CRAC channel activation (Fig. 1A). Orai1 contains intracellular N- and C-termini and four transmembrane segments and assembles as a hexameric complex forming a central Ca^{2+}-permeating pore in the PM, whereas Stim1 is a single membrane-spanning protein present in the ER membrane and uses an EF-hand Ca^{2+}-binding motif in the ER-facing part as the Ca^{2+} sensor. The CRAC channel is activated through the so-called diffusion trap mechanism [16]. Namely, the reduction in ER Ca^{2+} promotes Stim1 to aggregate and translocate (diffuse) to the PM-ER junction, where Stim1 binds to Orai1 and induces the channel

to open, allowing extracellular Ca^{2+} to enter the cytosol and then re-fill the ER via the SERCA. Orai1 and Stim1 are the most common components of the SOC channel in many cell types. Nonetheless, there is increasing evidence to show that the other two members of the Orai family, Orai2 and Orai3, working together with Stim1 or Stim2, can also form CRAC channels, independently of or via heteromerizing with Orai1, that have some distinctive differences in pharmacological properties [99–102].

4. Role of the SOC/CRAC channel in ATP-induced Ca^{2+} signalling in MSC

The first experimental evidence suggesting the SOC channel being an integral part of the ATP-induced Ca^{2+} signalling mechanism in MSC was from an early study by Kawano *et al.* [75] examining the molecular mechanisms underpinning the spontaneous oscillations or periodic increases in the $[Ca^{2+}]_i$ in human bone marrow-derived MSC (BM-MSC) observed under *in vitro* culture conditions. Such Ca^{2+} oscillations were ablated by treatment with the generic P2 receptor antagonist PPADS or the PLC inhibitor U73122, as well as 2-aminoethoxydiphenyl borate (2-APB) known to block the IP_3R and SOC channel. The Ca^{2+} oscillations and ATP in the culture medium were also obliterated by treatment with hexokinase together with glutamate, a combination known to consume ATP, and by treatment with octanol, palmitoleic acid or 18α-glycyrrhetinic acid (AGA), all of which are known to block the hemi-gap junction channel. Furthermore, the Ca^{2+} oscillations were lost after treatment with BzATP or APPS, both of which can block the $P2Y_1$ receptor. Collectively, these observations led to the proposal of a mechanism generating the spontaneous Ca^{2+} oscillations, in which ATP is spontaneously released into the extracellular space, through the hemi-gap junction channel, and activates the $P2Y_1$-$G_{q/11}$-PLC-IP_3R pathway to release the ER Ca^{2+} and induce SOCE [75]. Both ER Ca^{2+} release and SOCE, albeit differing spatiotemporally, contribute to the increase in the $[Ca^{2+}]_i$. The molecular identity of the SOC channel however was not determined in the study. Riddle and colleagues proposed ATP release and subsequent activation of the P2Y-$G_{q/11}$-PLC-IP_3R pathway to trigger ER Ca^{2+} release as an important mechanism resposnbile for the rise in the $[Ca^{2+}]_i$ in human BM-MSC in response to oscillatory flow fluid-induced shear stress [54, 77]. They demonstrated the expression of $P2Y_2$ and $P2Y_{11}$, and also P2X7, but not $P2Y_1$, using western blotting or immunocytochemistry, but did not examine in detail the exact roles of these receptors and the SOC channel in fluid flow-induced ATP-mediated Ca^{2+} signalling. Interestingly, it was shown that fluid flow-induced ATP release was insensitive to AGA but was considerably suppressed by treatment with monensin, which is known to prevent vesicle budding from the Golgi apparatus, or N-ethylmaleimide, which is known to block vesicle fusion with the PM. These observations suggest that BM-MSC releases ATP in response to fluid flow through a vesicular mechanism [77], rather than through the hemi-gap junction channel initially proposed to mediate spontaneous ATP release [75].

We have examined in a recent study the expression of the SOC channel as well as the P2X and P2Y receptors and their roles in mediating ATP-induced Ca^{2+} signalling in human dental pulp derived MSC (DP-MSC) [80]. We showed using the "Ca^{2+} add-back" experimental protocols that depletion of the ER Ca^{2+} by treatment with TG induced strong SOCE (Fig. 1B). Furthermore, TG-induced SOCE was reduced by treatment with 2-APB, or syntha 66, a SOC channel selective inhibitor. These results clearly support the expression of the SOC channel in human DP-MSC [80]. In human DP-MSC, exposure to exogenous ATP also induced strong but transient Ca^{2+} responses in extracellular Ca^{2+}-free solutions, indicating release of the ER Ca^{2+} as a result of ATP-induced activation of the P2Y-$G_{q/11}$-PLC-IP_3R pathway. In addition, we have shown that ADP, a $P2Y_1$ selective agonist, and BzATP, an agonist for the $P2Y_{11}$ receptor (and also for the P2X receptors), were effective in inducing Ca^{2+} responses in extracellular Ca^{2+}-containing solutions [80]. ATP-induced Ca^{2+} response was significantly attenuated by treatment with 2-APB or syntha 66, as well as by treatment with PPADS or AZ11634737, a P2X7 receptor specific antagonist. Consistently, the mRNA transcripts of P2X7, $P2Y_1$ and $P2Y_{11}$, but not $P2Y_2$, were consistently detected in human DP-MSC, using reverse transcription-polymerase chain reaction (RT-PCR). Furthermore, ATP-induced Ca^{2+} responses were reduced after treatment with small interference RNA (siRNA) that specifically knocked down the expression of P2X7, $P2Y_1$ or $P2Y_{11}$. Taken together, these results support participation of the SOC channel, in addition to the P2X7, $P2Y_1$ and $P2Y_{11}$ receptors, in mediating ATP-induced Ca^{2+} signalling (Fig. 1A) [80]. Two recent studies, one using human adipose tissue-derived MSC (AT-MSC) [97] and the other using rat DP-MSC [82], have also shown that exposure to exogenous ATP induced Ca^{2+} responses in the absence, as well as in the presence, of extracellular Ca^{2+}. ATP-induced Ca^{2+} response was inhibited by treatment with TG, U73122 or 2-APB, consistently supporting a critical role of ER Ca^{2+} release following activation of the P2Y-$G_{q/11}$-PLC-IP_3R pathway in ATP-induced Ca^{2+} signalling. One study has proposed, based on the pharmacological profile, $P2Y_2$ as the receptor mediating ATP-induced Ca^{2+} signalling [97], and the other study did not identify the P2Y receptor(s) involved [82]. None of these studies have determined the role of the SOC channel, or the contribution of SOCE, which would occur following release of ER Ca^{2+}, in ATP-induced Ca^{2+} signalling.

As the CRAC channel made of Orai1 and Stim1 represents the SOC channel with the best-established protein components and activation mechanism, we have fur-

ther examined the expression of Orai1, Stim1 and Stim2, and their roles in ATP-induced Ca^{2+} signalling in human DP-MSC [80]. The mRNA expression for Orai1, Stim1 and Stim2, in human DP-MSC was detected using RT-PCR. Importantly, TG-induced SOCE was reduced by siRNA-mediated knockdown of the expression of Orai1 or Stim1, but not Stim2, supporting that Orai1 in pairs with Stim1 forms the CRAC channel [80]. Moreover, consistent with the inhibition by syntha 66, which has recently been shown as an Orai1-specific CRAC channel inhibitor [102], ATP-induced Ca^{2+} response was suppressed by siRNA-mediated reduction of the expression of Orai1 or Stim1 (Fig. 1C). These results provide the first line of evidence to show that Orai1 and Stim1 constitute the CRAC channel as a significant mechanism contributing in ATP-induced Ca^{2+} signalling.

In summary, accumulating evidence supports the SOC channel, particularly the CRAC channel made of Orai1 and Stim1, as an integral part of the mechanism for ATP-induced Ca^{2+} signalling in MSC.

5. Role of the SOC/CRAC channel in ATP-induced regulation of MSC function

Studies have shown that extracellular ATP, applied exogenously or released by MSC, can regulate MSC differentiation, proliferation and migration. Moreover, these studies have gathered substantial evidence to support that both P2X7 and $G_{q/11}$-coupled P2Y receptors and their downstream Ca^{2+}-dependent signal pathways play a signficant role in such ATP-induced regulation of MSC functions (reviewed by [103]). In contrast, the role of the SOC or CRAC channel in ATP-induced regulation of MSC function, despite being implied, still remains elusive.

In the study examining the molecular mechanisms underlying the spontaneous Ca^{2+} oscillations in human BM-MSC, Kawano *et al.* [75] noticed that the spontaneous Ca^{2+} oscillations disappeared after induction of differentiation to adipocytes. They also showed that such Ca^{2+} signaling was critical for the translation from the cytosol to the nucleus of nuclear factor of activated T-cells (NFAT), a vital transcription factor driving the expression of many genes. However, it is still unknown regarding the mechanisms underlying the contribution of such spontaneously occurring Ca^{2+} signalling, with SOCE being part of it, in NFAT activation and, furthermore, in adipogenesis. The recent study by Stovall *et al.* [82] has shown that exposure of rat DP-MSC to exogenous ATP stimulated osteoblast formation and the expression of multiple osteogenic genes. As discussed above, the study has proposed the $G_{q/11}$-coupled P2Y receptor as the major ATP receptor in rat DP-MSC, leading to the conclusion that ATP enhances osteogenic differentiation via $G_{q/11}$-coupled P2Y receptor-dependent Ca^{2+} signalling. However, the role of the SOC channel-mediated Ca^{2+} signalling in such ATP-induced regulation of osteogenesis remains unknown. At this point, it is worth mentioning that several other recent studies using human MSC preparations from several tissues provide evidence to show that the P2X7 receptor also plays a significant role in ATP-induced regulation of osteogenic differentiation [68, 69, 79, 81].

In the above-discussed studies revealing that fluid flow evoked Ca^{2+} signalling through ATP release and activation of the $P2Y_2/P2Y_{11}$-$G_{q/11}$-PLC-IP_3R pathway to cause ER Ca^{2+} release in human BM-MSC, Riddle *et al.* [54, 77] also demonstrated that fluid flow enhanced cell proliferation. Furthermore, they showed that fluid flow stimulated the activity of protein kinase C (PKC) and downstream signalling molecules, MEK and ERK1/2 mitogen-activated protein kinases, as well as calcineurin, a Ca^{2+}/calmodulin-dependent phosphatase. Consistent with the well-established roles of these Ca^{2+}-dependent signal pathways in the regulation of cell proliferation, fluid flow-induced stimulation of cell proliferation was inhibited by treatment with the MEK/ERK inhibitor U-0126 or the calcineurin inhibitor cyclosporine A [54]. Moreover, fluid flow-induced activation of calcineurin and stimulation of cell proliferation, as well as fluid flow-induced increase in the $[Ca^{2+}]_i$, were inhibited by treatment with apyrase, supporting a critical role of ATP release and induction of intracellular Ca^{2+} signalling and activation of downstream Ca^{2+}-dependent signal pathways [77]. Like ATP released by fluid flow, exposure to exogenous ATP, but not ADP, AMP and adenosine, the major ATP metabolites, significantly stimulated cell proliferation. Taken together, these results provide clear evidence to show that fluid flow stimulates MSC proliferation via inducing ATP release and activation of the $G_{q/11}$-coupled P2Y receptors, leading to ER Ca^{2+} release and activation of the downstream Ca^{2+}-dependent signal pathways. As pointed above, it was anticipated that SOCE occurred following ER Ca^{2+} release under these conditions. It is interesting to investigate the role of the SOC channel, particularly the Orai1/Stim1 CRAC channel, in participating in fluid flow-induced ATP-mediated Ca^{2+} signalling and regulation of cell proliferation.

In our recent study we have shown that exposure to exogenous ATP stimulated human DP-MSC migration and provided evidence to support a significant role of the Orai1/Stim1 CRAC channel, in addition to the $P2Y_1$, $P2Y_{11}$ and P2X7 receptors, in mediating ATP-induced stimulation of cell migration [80]. ATP-induced stimulation of cell migration was not affected by treatment with CGS1593, an adenosine receptor antagonist, consistent with no critical involvement of ATP metabolites in ATP-induced cell migration, as discussed above in fluid flow/ATP-induced cell proliferation. ATP-induced stimulation of cell migration was suppressed by treatment with 2-APB and also ablated by siRNA-mediated knockdown of the expression of Orai1 or Stim1, as well as knockdown of the expression of $P2Y_1$, $P2Y_{11}$ or P2X7. More-

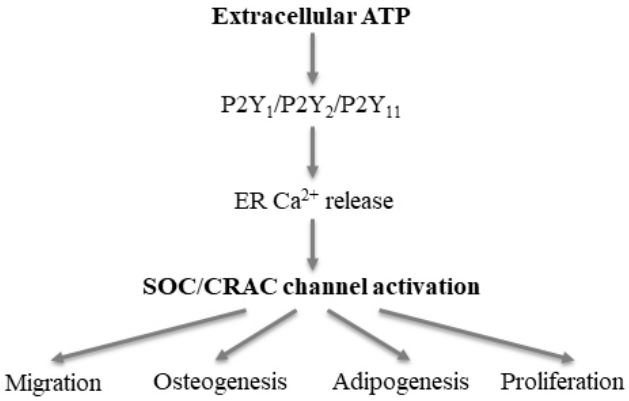

Fig. 2. Proposed roles of the SOC/CRAC channel in ATP-induced regulation of mesenchymal stem cell (MSC) function. Extracellular ATP activates the $P2Y_1$, $P2Y_2$ and/or $P2Y_{11}$ receptor that leads to Ca^{2+} release from the endoplasmic reticulum (ER), which in turn activates the store-operated Ca^{2+} (SOC)/Ca^{2+}-release-activated Ca^{2+} (CRAC) channel and results in extracellular Ca^{2+} entry (illustrated in Fig.1A). Such a mechanism in MSC has been shown to play a signficant role in ATP-induced regulation of cell migration or implied in ATP-induced ostogenesis, adipogenesis and proliferation (see text for further details).

over, in a more recent study, we have shown that ATP-induced cell migration was largely inhibited by treatment with PF431396, an inhibitor of PYK2, a Ca^{2+}-sensitive tyrosine kinase which is a member of the focal adhesion kinase family, or treatment with U0126 to inhibit MEK/ERK, which is known to be activated downstream of PYK2 [83]. Collectively, our studies support that intracellular Ca^{2+} signalling, generated via the $G_{q/11}$-coupled $P2Y_1$/$P2Y_{11}$ receptors and Ora1/Stim1 CRAC channel, as well as the P2X7 receptor, and subsequent activation of downstream Ca^{2+}-dependent signal pathways are important in driving ATP-induced stimulation of MSC migration. Furthermore, consistent with human MSC releasing ATP in response to mechanical signals, we have presented evidence to show that the mechanosensitive Piezo1 channel is expressed in human DP-MSC, and its activation promotes cell migration that critically depends on ATP release and activation of the P2 receptor, PYK2 and MEK/ERK [83]. These results have led us to propose that ATP as an extracellular signal can induce Ca^{2+} signalling to stimulate MSC migration, through activation of the $P2Y_1$/$P2Y_{11}$-$G_{q/11}$-PLC-IP_3R pathway that results in ER Ca^{2+} release and subsequent Orai1/Stim1 CRAC channel-mediated SOCE, in addition to Ca^{2+} influx through the P2X7 receptor.

In summary, emerging evidence supports the SOC/CRAC channel in MSC to be important in ATP-induced regulation of cell migration, but more investigations are required to understand the role of the SOC/CRAC channel in ATP-induced regulation of cell proliferation and differentiation (Fig. 2).

6. Concluding remarks

Extracellular ATP has been shown as an autocrine/paracrine signal that induces Ca^{2+} signaling in MSC via the P2X receptors that mediate Ca^{2+} influx and/or the $G_{q/11}$-coupled P2Y receptors that lead to ER Ca^{2+} release to stimulate Ca^{2+}-dependent downstream signal pathways and thereby regulates cell proliferation, migration and differentiation. The reduction of ER Ca^{2+} further activates the SOC channel, a distinctive Ca^{2+} influx mechanism that is widely documented in mammalian cells. Emerging evidence supports the SOC channel, or more specifically, the Orai1/Stim1 CRAC channel, as an important mechanism that participates in ATP-induced Ca^{2+} signalling in MSC and ATP-induced regulation of cell function. Nonetheless, compared to the P2X and P2Y receptors, the SOC/CRAC channel in terms of its contribution to ATP-induced Ca^{2+} signalling and regulation in MSC function remains less well understood. As discussed above, MSC exhibit a high sensitivity to diverse mechanical signals that regulate multiple MSC functions. This attribute is of particular importance to the translational applications of MSC, considering mechanically different scaffolds used in tissue engineering that may affect cell viability, proliferation, migration and differentiation. The interactions of MSC with extracellular matrix and recipient tissues may also influence their ability of migration and tissue homing, a well-recognised factor limiting the efficacy of MSC-based therapies. Interestingly, increasing evidence supports ATP release and activation of the P2 receptors, particularly the $G_{q/11}$-coupled P2Y receptors, as a mechanism converting mechanical signals to Ca^{2+} signals in the regulation of cell functions [93]. More research efforts are clearly required to better understand the role of the SOC/CRAC channel in ATP-induced Ca^{2+} signalling in MSC and regulation of cell function by physical, chemical and biological stimuli or signals known to induce ATP release and activation of the P2Y-$G_{q/11}$-PLC-IP_3R pathway. Such information is useful not only to the utilisation of MSC in regenerative medicines but also to the improvement of our knowledge about basic MSC biology.

7. Author contributions

LHJ initiated the discussion and drafted the manuscript. LW, SR and XBY contributed to the discussion and revised the manuscript. All authors approved the manuscript.

8. Ethics approval and consent to participate

Not applicable.

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Not applicable.

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11. Conflict of interest

The authors declare no conflict of interest.

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