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SPOTLIGHT

# When huffing and puffing $Ca^{2+}$ goes global, breast cancer cells are unmoved

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**In this issue, Militsin et al. (https://doi.org/10.1083/jcb.202411203) reveal how STIM1 and STIM2—beyond their typical role as ER $Ca^{2+}$ sensors that activate Orai1—control $IP_3R$-mediated $Ca^{2+}$ dynamics, thereby regulating breast cancer cell migration and invasion.**

$Ca^{2+}$ signaling mediates numerous cell functions, including cell proliferation and migration, and as such plays a dominant role in many aspects of cancer (1). The canonical receptor-evoked $Ca^{2+}$ signal is initiated by activation of PLC that hydrolyzes $PI(4,5)P_2$ to $IP_3$ and diacylglycerol. $IP_3$ opens $IP_3$ receptors ($IP_3Rs$), releasing $Ca^{2+}$ stored in the ER to increase cytoplasmic $Ca^{2+}$ ($[Ca^{2+}]_i$) rapidly. $Ca^{2+}$ release from the ER causes unfolding and homo or hetero dimerization of the ER $Ca^{2+}$ sensors STIM1 and STIM2. STIM1 recruits and activates primarily the $Ca^{2+}$ influx channel Orai1. $Ca^{2+}$ influx sustains the intracellular $Ca^{2+}$ signal and provides the $Ca^{2+}$ needed to replenish the ER $Ca^{2+}$ store. In addition to activating Orai1, STIM proteins tether the ER and PM to form and stabilize junctions between them. In fact, the ER/PM junctions are where $Ca^{2+}$ signaling complexes are formed and the $Ca^{2+}$ signal is initiated and regulated (2, 3). The function of all pathways generating the receptor-evoked $Ca^{2+}$ signal, including STIM1 and STIM2, are involved in the classical and emerging hallmarks of cancers (1).

Physiologically, most $Ca^{2+}$ signals are in the form of repetitive short $Ca^{2+}$ increases called $Ca^{2+}$ puffs that are confined to cellular domains and which only infrequently globalize to cause a large and sustained increase in $[Ca^{2+}]_i$. $Ca^{2+}$ puffs involve repetitive transient activation of $IP_3Rs$ to transiently release $Ca^{2+}$ from the ER; the frequency of $Ca^{2+}$ puffs is determined by the strength of cell stimulation, and thus the level of $IP_3$. Confined $Ca^{2+}$ puffs are common in polarized cells like exocrine, neuronal, and muscle cells, as well as at the leading edge of migrating cells (4, 5). However, the mechanism of transition from $Ca^{2+}$ puffs to global $Ca^{2+}$ signals is not well understood, nor is the role of each form of the $Ca^{2+}$ signal patterns in cell proliferation, migration, and tumorigenesis. A study in this issue by Militsin et al. (6) provides fresh insight on these two imperative questions.

STIM1 and STIM2 play a unique role in $Ca^{2+}$ signaling by mediating the communication between the cell surface (PM) and cell interior (ER) and by dynamically regulating cell signaling hubs at the ER/PM junctions (7, 8). Militsin et al. examined the role of STIM1 and STIM2 in the MDA-MB-231 breast cancer cell line by generating $STIM1^{-/-}$ (S1KO), $STIM2^{-/-}$ (S2KO), and $STIM1^{-/-}$+$STIM2^{-/-}$ double knockout (S1/S2DKO) cancer cells. Measuring *in vitro* cell proliferation and colony formation and *in vivo* tumor growth revealed that both STIMs are required for these activities. However, a surprising observation was made when comparing the effects of S1KO, S2KO, and S1/2DKO on cell adhesion, cell migration, and focal adhesion. While S1KO and S2KO cells showed reduced activity, S1/2DKO cells showed normal cell adhesion, migration, and focal adhesion. Interestingly, cell migration and focal adhesion indicate formation of polarized cell domains, which are controlled by dynamic ER/PM junction gradients (9), in which the STIM isoforms have critical roles (7, 8).

These findings not only reveal the participation of STIM isoforms in breast cancer initiation, migration, and invasion but also raise the question of why removing the two STIM isoforms restored, rather than eliminated, their involvement in these activities. Both pharmacological inhibitors and genetic modifications demonstrated that the unexpected results in S1/2DKO cells were due to altered $IP_3R$ function (6). Moreover, analysis of the pattern of $Ca^{2+}$ signals evoked by $IP_3R$ activation in cells expressing the various STIM combinations revealed a novel mechanism of STIM-mediated $IP_3R$ gating. Additionally, the $Ca^{2+}$ signal pattern had a novel and remarkable role in cell migration and cancer invasion (6). As illustrated in Fig. 1, controlled $IP_3R$ activation in WT breast cancer cell lines resulted in periodic $Ca^{2+}$ puffs that never globalized. By contrast, similar $IP_3R$ activation in S1KO and S2KO resulted in $Ca^{2+}$ puffs that rapidly globalized to generate a diffuse $Ca^{2+}$ pattern. Notably, $IP_3R$ activation in S1/2DKO resulted in periodic $Ca^{2+}$ puffs that never globalized. Overall, the findings by Militsin et al. provide strong support that STIM1 and STIM2 facilitate the opening of $IP_3Rs$, in addition to their well-established role in opening Orai channels. By opening $IP_3R$, STIMs set the $Ca^{2+}$ signaling pattern to either $Ca^{2+}$ puffs or

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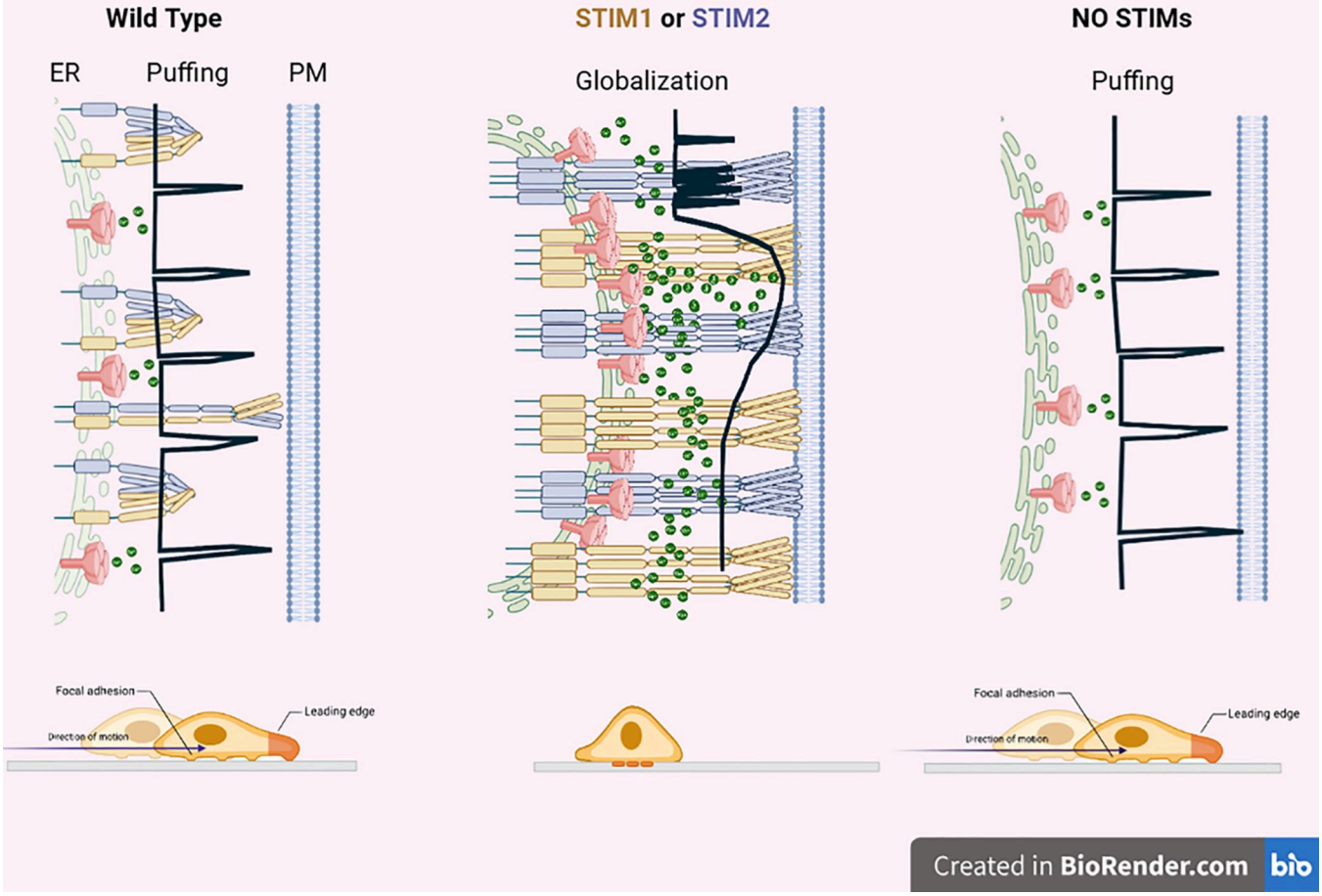


Figure 1. **STIM1 and STIM2 gate $IP_3Rs$ opening.** The model depicts the pattern of the $Ca^{2+}$ signal observed in the presence and absence of the STIM isoforms during controlled increase in $IP_3$. A limited and local release of $IP_3$ evokes $Ca^{2+}$ puffs that never globalize (left panel). Deletion of either STIM1 or STIM2 partially reduced ER $Ca^{2+}$ level, and the same $IP_3$ stimulus causes the $Ca^{2+}$ puffs to globalize into a diffuse $Ca^{2+}$ signal (middle panel). Surprisingly, in the absence of both STIM1 and STIM2, the $IP_3$-mediated $Ca^{2+}$ puffs never globalize (right panel). In breast cancer cells, $Ca^{2+}$ puffs stimulate cell migration and invasion, while diffuse $Ca^{2+}$ signals restrict cell migration and tumorigenesis.

a diffuse global increase in $[Ca^{2+}]_i$. In cancer, the $Ca^{2+}$ puff pattern supports cell migration and invasion, while global $[Ca^{2+}]_i$ induction restricts cell migration.

The findings by Militsin et al. also raise the key questions of how STIMs regulate $IP_3Rs$ and why $Ca^{2+}$ signaling in S1/2DKO cells does not globalize. The authors suggest STIMs indirectly regulate $IP_3R$ gating since the two proteins could not be co-immunoprecipitated in MDA-MB-231 cell lysates. However, co-immunoprecipitation of native STIM1-$IP_3Rs$ and FRET between the expressed proteins as part of a signaling complex has been reported in several studies (for example, [3]). Therefore, modification of $IP_3R$ function by the N terminus of STIM1 and STIM2, which has been described in colorectal cancer (10), may partly account for STIM-mediated $IP_3R$ gating. An additional mechanism can be $IP_3R$ sensitization by $PI(4,5)P_2$, which facilitates $Ca^{2+}$ release (11). STIMs assemble $Ca^{2+}$ signaling complexes at $PI(4,5)P_2$-rich domains that may be necessary for $PI(4,5)P_2$-mediated sensitization of $IP_3Rs$ and globalization of the $Ca^{2+}$ signal. Finally, the most vital mechanism may be the control of ER $Ca^{2+}$ content by STIMs. ER $Ca^{2+}$ content controls STIM clustering (7) and $IP_3R$ activity (12), whereas STIM deletion reduces ER $Ca^{2+}$ content (6). Reduced ER $Ca^{2+}$ would, therefore, be expected to cluster STIM1 and STIM2 homodimers at the ER/PM junctions to facilitate and enhance $IP_3R$-mediated $Ca^{2+}$ release and globalize the $Ca^{2+}$ signal (Fig. 1, middle).

Altered $IP_3R$ clustering may account for the puzzling observations in S1/2DKO cells. $IP_3$-mediated $Ca^{2+}$ release increases with increased $IP_3R$ clustering (4). $IP_3R$ clustering takes place at the ER/PM junctions (3), and STIM proteins have a major role in ER/PM junction formation and stabilization (7, 8). In S1/2DKO cells, the complete absence of STIM proteins reduces ER/PM junction formation, thereby preventing $IP_3R$ clustering. This mechanism may underlie the failure of $Ca^{2+}$ puffs to globalize in complete STIM deficiency (Fig. 1, right), although other mechanisms may also contribute. Considering the critical role of STIM in regulation and shaping $Ca^{2+}$ signal patterns in cancer and the observation that STIM expression changes during tumorigenesis (10), it is important and clinically relevant to further explore the mechanisms by which STIMs gate $IP_3Rs$ to shape $Ca^{2+}$ signals.

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