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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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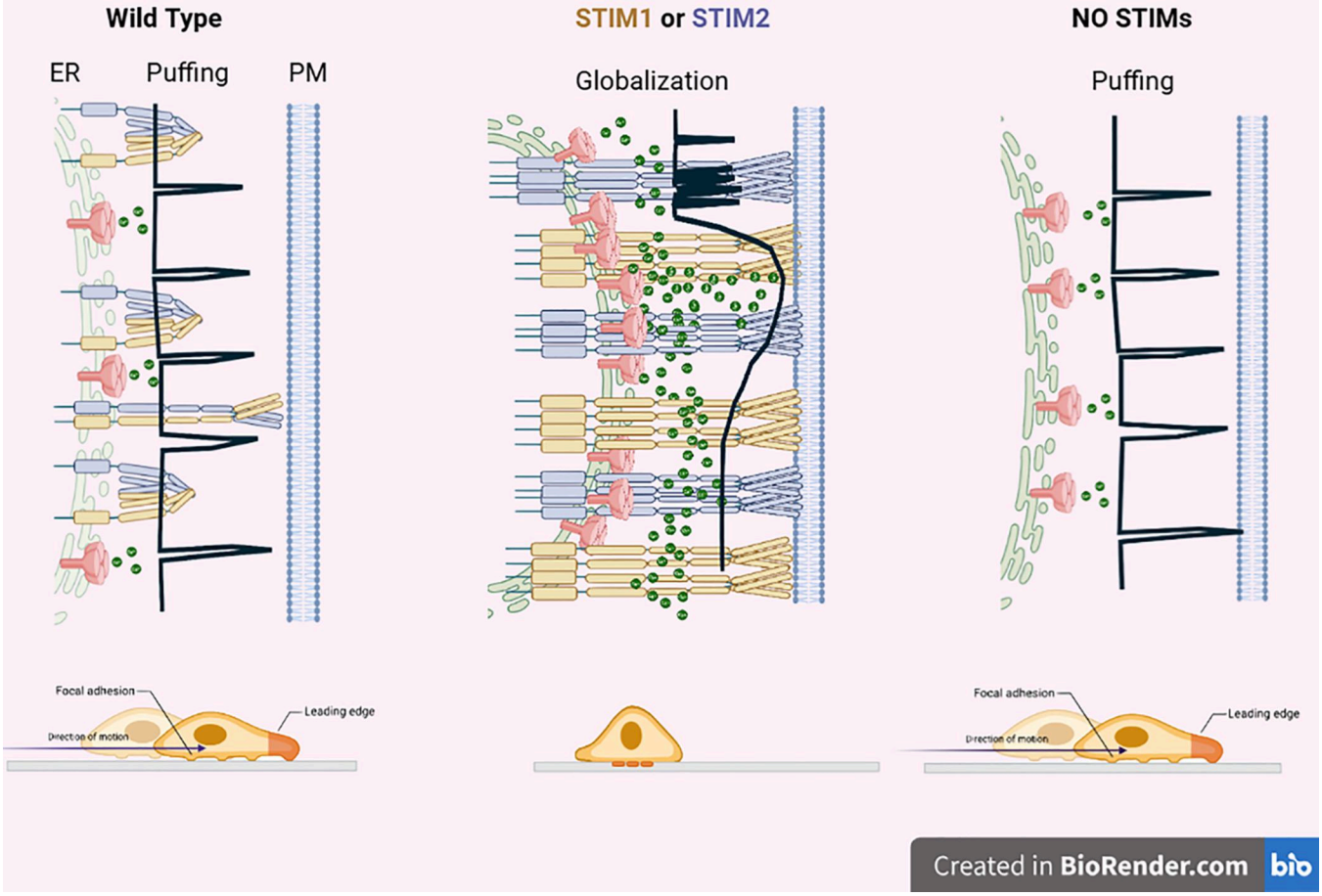

Figure 1. **STIM1 and STIM2 gate IP_3Rs opening.** The model depicts the pattern of the Ca^{2+} signal observed in the presence and absence of the STIM isoforms during controlled increase in IP_3. A limited and local release of IP_3 evokes Ca^{2+} puffs that never globalize (left panel). Deletion of 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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