Disseminating melanoma cells surf on calcium waves

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Abbreviations: ECM, extracellular matrix; HCC, hepatocellular carcinoma; MT1-MMP, membrane-type 1 matrix metalloprotease; SOCE, store-operated calcium entry.

Dysregulated calcium signaling has been increasingly implicated in tumor dissemination and progression. In a recent study, we investigated the mechanism underlying calcium-mediated invasion and metastasis in melanoma and discovered that hyperactive Ca\textsuperscript{2+} oscillation in melanoma cells enhanced invasion and metastasis by promoting invadopodium formation and extracellular matrix remodeling.

Gaining invasiveness and overcoming the physical barriers imposed by the extracellular matrix (ECM) are the first, and arguably most critical, steps of tumor metastasis. In addition to facilitating dissemination, remodeling of the ECM is essential for tumor growth and the establishment of metastatic niches by malignant cells. Invadopodia of malignant cancer cells are actin-rich, ECM-degradative membrane protrusions that are critical for tumor invasion and metastasis. In a recent study, we investigated the regulation of invadopodium formation and metastasis by Ca\textsuperscript{2+} signaling in melanoma. We unexpectedly discovered that hyperactive Ca\textsuperscript{2+} signaling in metastatic melanoma cells is organized in the form of oscillatory waves to orchestrate invadopodium assembly and melanoma invasion.

In recent years, dysregulated Ca\textsuperscript{2+} signaling has been increasingly implicated in cancer invasion and metastasis, yet the underlying mechanism remained largely unclear. To gain mechanistic insight into Ca\textsuperscript{2+}-mediated invasion and metastasis, we examined the role of Ca\textsuperscript{2+} signaling in invadopodium formation in melanoma cells and discovered that blocking store-operated calcium (SOC) channel signaling significantly decreased invadopodium number and activity. Accompanying the assembly of invadopodia was an oscillatory Ca\textsuperscript{2+} signal mediated by the SOC channel proteins STIM1 and Orai1. Interestingly, disruption of the Ca\textsuperscript{2+} oscillation by either blocking store operated calcium entry (SOCE; which decreased cytosolic Ca\textsuperscript{2+} concentration), or by inducing constitutive calcium entry with thapsigargin or the ionophore A-23187 (which increased cytosolic Ca\textsuperscript{2+} concentration) similarly inhibited invadopodium assembly and melanoma invasion, signifying the importance of temporal Ca\textsuperscript{2+} signal coding during metastatic dissemination.

By screening a panel of protein kinases, we identified the non-receptor tyrosine kinase Src as a downstream effector of SOCE. The notion that SOCE regulates invadopodium assembly through Src is further supported by the rescue of defective invadopodium formation in STIM1 knockout melanoma cells by constitutively active v-Src, and the abrogation of STIM1-mediated invadopodium assembly by the Src inhibitor dasatinib. The fact that constitutive Ca\textsuperscript{2+} influx induced by thapsigargin and A-23187 was a robust activator of Src begs the question: Why do melanoma cells use an oscillatory Ca\textsuperscript{2+} signal instead of a steady Ca\textsuperscript{2+} increase?

Ca\textsuperscript{2+} is a notoriously versatile second messenger. It is estimated that hundreds of genes in the human genome contain Ca\textsuperscript{2+} binding EF-hand or C2 domains. The specificity and versatility of Ca\textsuperscript{2+} signaling relies on the intricate spatial and temporal coding of cytosolic Ca\textsuperscript{2+} concentration. By compartmentalizing Ca\textsuperscript{2+} signals into spatial-temporal patterns, cells are able to activate selective downstream signaling events at a defined time and subcellular location. It is believed that the frequency and amplitude of Ca\textsuperscript{2+} oscillations serve as digital signals that selectively activate threshold-dependent downstream events. The tight control of cytosolic Ca\textsuperscript{2+} is critical not only for signaling specificity but also for cell survival, since a prolonged and uncontrolled global increase in cytosolic Ca\textsuperscript{2+} is toxic to the cell and eventually leads to cell death. By organizing SOCE signals in the form of Ca\textsuperscript{2+} oscillation, melanoma cells are able to...
provide the Ca\textsuperscript{2+} signal necessary for invadopodium assembly and ECM remodeling over an extended period of time without causing cytotoxicity (Fig. 1A). In contrast, although constitutive Ca\textsuperscript{2+} influx induced by thapsigargin and A23187 robustly increases Src activity it might also indiscriminately activate hundreds of other Ca\textsuperscript{2+}-dependent signaling pathways, which eventually reduce melanoma cell fitness and inhibit melanoma invasion (Fig. 1B).

It is also possible that melanoma invasion and invadopodium assembly require a coordinated cycle of Ca\textsuperscript{2+} peaks and valleys, as recently demonstrated in mast cell exocytosis by Wollman and Meyer.\textsuperscript{8} Ca\textsuperscript{2+} oscillation in antigen-activated mast cells drives the cyclic assembly and disassembly of cortical actin. Newly assembled cortex actin serves as a carrier to capture secretory vesicles, whereas disassembly of cortical actin allows the passage of vesicles to facilitate membrane fusion. Intriguingly, we discovered that recycling of membrane type 1 matrix metalloprotease (MT1-MMP, also known as MMP14) to the plasma membrane required SOCE, blockage of which resulted in entrapment of MT1-MMP in endosomes.\textsuperscript{3} It would be interesting to determine whether SOCE-mediated Ca\textsuperscript{2+} oscillation coordinates the recycling of endocytosed MT1-MMP to the plasma membrane.

Of note, tumor-promoting Ca\textsuperscript{2+} oscillation has also recently been observed in esophageal squamous cell carcinoma and hepatocellular carcinoma (HCC).\textsuperscript{9,10} Overexpression of Orai1 in esophageal carcinoma cells is responsible for hyperactive Ca\textsuperscript{2+} oscillation, which promotes cancer cell motility and proliferation \textit{in vitro} and tumor growth in a xenograft model.\textsuperscript{10} In HCC the voltage-gated calcium channel subunit \textit{CACNA2D1} was found to be a marker for recurrent disease. Recurrent HCC cells had higher expression of \( \alpha_{2} \delta_{1} \) and hyperactive Ca\textsuperscript{2+} oscillation, which could be inhibited by a blocking antibody targeting \textit{CACNA2D1}.\textsuperscript{10} These observations, together with our recent finding, suggest that Ca\textsuperscript{2+} oscillation might be a signaling mechanism that is commonly hijacked by malignant cells to facilitate cancer progression. Future investigation in this area will likely significantly advance our understanding of how deregulated Ca\textsuperscript{2+} signaling promotes cancer malignancy.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Figure 1. Regulation of invadopodia by Ca\textsuperscript{2+} oscillation. (A) Hyperactive store-operated calcium entry (SOCE) increases Ca\textsuperscript{2+} oscillation frequency and amplitude, which selectively activates Src to promote invadopodium assembly and extracellular matrix remodeling. (B) Disruption of Ca\textsuperscript{2+} oscillation, either by a constitutive increase in cytosolic Ca\textsuperscript{2+} or by blockage of SOCE, inhibits invadopodium formation and melanoma invasion.
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