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Disseminating Melanoma Cells Surf on Calcium Waves

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Abstract

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

Gaining invasiveness and overcoming physical barrier imposed by the extracellular matrix is the first and arguably most critical steps of tumor metastasis (1). In addition to facilitating dissemination, remodeling of the extracellular matrix (ECM) is essential for tumor growth and the establishment of metastatic niches by malignant cells (2). Invadopodia in malignant cancer cells are actin-rich, ECM degrading membrane protrusions critical for tumor invasion and metastasis (1). In a recent study, we investigated the regulation of invadopodium formation and melanoma metastasis by Ca^{2+} signaling (3). We unexpectedly discovered that hyperactive Ca^{2+} signal in metastatic melanoma cells is organized in the form of oscillatory waves to orchestrate invadopodial assembly and melanoma invasion (3).

In recent years, dysregulated Ca^{2+} signaling has been increasingly implicated in cancer invasion and metastasis, and yet, the underlying mechanism was largely unclear (4, 5). To gain mechanistic insight underlying Ca^{2+}-mediated invasion and metastasis, we examined the role of Ca^{2+} signaling in invadopodium formation in melanoma cells, and discovered that blocking store-operated calcium channel signaling significantly decreased invadopodium number and activity. Accompanying the assembly of invadopodia was oscillatory Ca^{2+} signal, mediated by SOC channel proteins *STIM1* and *ORAI1*. Interestingly, disruption of the Ca^{2+} oscillation by either blocking store operated calcium entry (SOCE) (which decreased cytosolic Ca^{2+} concentration), or by inducing constitutive calcium entry with thapsigargin or ionophore A-23187 (which increased cytosolic Ca^{2+} concentration) similarly inhibited invadopodium assembly and melanoma invasion, signifying the importance of temporal Ca^{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 invadopodium formation defect in *STIM1* knockdown melanoma cells by constitutively active v-Src, and the abrogation of *STIM1*-mediated invadopodium assembly by Src inhibitor dasatinib. Since constitutive Ca^{2+} influx

induced by thapsigargin and A-23187 was a robust activator of Src, this begs the question: why did melanoma cells use oscillatory Ca^{2+} signal instead of steady Ca^{2+} increase?

Ca^{2+} is a notoriously versatile second messenger. It is estimated that hundreds of genes in the human genome contain Ca^{2+} binding EF-hand or C2 domains (6). The specificity and versatility of Ca^{2+} signaling relies on the intricate spatial and temporal coding of cytosolic Ca^{2+} concentration (6). By compartmentalizing Ca^{2+} signals into spatial-temporal patterns, cells are able to activate selective downstream signaling events at defined time and subcellular location (6). It is believed that the frequency and amplitude of Ca^{2+} oscillations serve as digital signals that selectively activate threshold-dependent downstream events. The tight control of cytosolic Ca^{2+} is not only critical for signaling specificity, but also for cell survival, since prolonged and uncontrolled global increase in cytosolic Ca^{2+} is toxic to the cell and eventually leads to cell death (7). By organizing SOCE signal in the form of Ca^{2+} oscillation, melanoma cells are able to provide the Ca^{2+} signal necessary for invadopodium assembly and ECM remodeling over an extended period of time without causing cytotoxicity (Fig. 1A). In contrast, constitutive Ca^{2+} influx induced by thapsigargin and A23187, although robustly increased Src activity, might also indiscriminately activate hundreds of other Ca^{2+}-dependent signaling pathways, which eventually reduced melanoma cell fitness and inhibited melanoma invasion (Fig. 1B) .

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

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

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Abbreviations

SOCE	store-operated calcium entry
ECM	extracellular matrix
MT1-MMP	membrane-type 1 matrix metallopretease
HCC	hepatocellular carcinoma\

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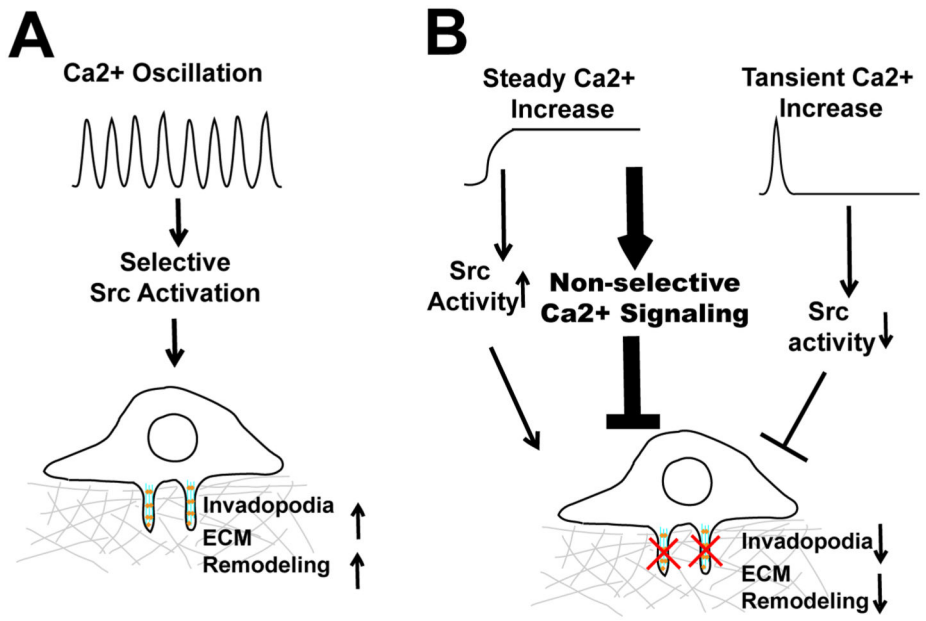

Figure 1.

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