The Regulation of Tumor Cell Invasion and Metastasis by Endoplasmic Reticulum-to-Mitochondrial Ca2+ Transfer

Carl White*

Physiology and Biophysics, Chicago Medical School, Rosalind Franklin University of Medicine and Science, North Chicago, IL, United States

Cell migration is one of the many processes orchestrated by calcium (Ca2+) signaling, and its dysregulation drives the increased invasive and metastatic potential of cancer cells. The ability of Ca2+ to function effectively as a regulator of migration requires the generation of temporally complex signals within spatially restricted microdomains. The generation and maintenance of these Ca2+ signals require a specific structural architecture and tightly regulated communication between the extracellular space, intracellular organelles, and cytoplasmic compartments. New insights into how Ca2+ microdomains are shaped by interorganellar Ca2+ communication have shed light on how Ca2+ coordinates cell migration by directing cellular polarization and the rearrangement of structural proteins. Importantly, we are beginning to understand how cancer subverts normal migration through the activity of oncogenes and tumor suppressors that impinge directly on the physiological function or expression levels of Ca2+ signaling proteins. In this review, we present and discuss research at the forefront of interorganellar Ca2+ signaling as it relates to cell migration, metastasis, and cancer progression, with special focus on endoplasmic reticulum-to-mitochondrial Ca2+ transfer.

Keywords: migration, Bcl-2, Bcl-XL, MCL1, reactive oxygen species, voltage-dependent anion channel, STIM, Orai

INTRODUCTION

Pathology is frequently associated with the dysregulation of intracellular calcium (Ca2+) signaling (1). Cancer is no exception, with many primary tumor cells and cell lines displaying aberrant expression of Ca2+ signaling genes (2, 3). While it is unlikely that somatic mutations affecting any one individual Ca2+ signaling gene are sufficient to drive tumorigenesis (4, 5), remodeling of the Ca2+ signal in cancer appears almost universal and confers survival advantages (3, 6). And so, it may be that dysfunctional Ca2+ signaling is indeed a determinant of tumorigenesis when coincident with cancer-driving oncogene and tumor suppressor mutations.

Also, many oncoproteins and tumor suppressor proteins can themselves directly modulate Ca2+ signaling. They achieve this, in large part, by interacting with Ca2+ channels, pumps, and exchangers localized at the plasma membrane and various intracellular compartments. The Bcl-2 family of oncoproteins has been the most extensively studied in this respect and found to regulate Ca2+ signaling in ways that complement their roles as apoptotic regulators, as recently reviewed (7). Similarly,
oncogenic Ras (8, 9) and the tumor suppressors promyelocytic leukemia (PML) (10), p53 (11), and BRCA1 (12) can all regulate apoptosis by impinging on the Ca\textsuperscript{2+} signal. Many of these proteins are enriched in spatially restricted domains created by the close apposition between the endoplasmic reticulum (ER) and the mitochondria, known as mitochondria-associated membranes (MAMs), where they function to modulate the flow of Ca\textsuperscript{2+} from the ER to mitochondria.

Ca\textsuperscript{2+} signaling also plays a role in cancer cell invasion and metastasis. Several different plasma membrane and ER-localized Ca\textsuperscript{2+} channels regulate the activity of effectors involved in motility and adhesion. Most of this regulation occurs by modifying the cytoplasmic Ca\textsuperscript{2+} signal and has been reviewed previously (13, 14). The significance of ER-mitochondrial Ca\textsuperscript{2+} communication in invasion and metastasis, however, has only recently emerged. This review will assess the literature relating to ER-mitochondrial Ca\textsuperscript{2+} communication. Our goal is to outline a theoretical framework that mechanistically links cancer-driven changes in ER-mitochondrial Ca\textsuperscript{2+} communication to its invasive and metastatic properties. We have made every attempt to include and reference original studies specifically related to this topic. When discussing a well-established concept, however, we direct readers to an appropriate review article.

**Ca\textsuperscript{2+} SIGNALING REGULATES MULTIPLE STEPS OF THE INVASION-METASTATIC CASCADE**

Tumor metastasis directly accounts for the vast majority of cancer deaths (15). Metastasis is characterized by a sequence of events known as the invasion-metastatic cascade (16, 17). During this process, cancer cells lose their attachment to other cells and the extracellular matrix (ECM), acquire migratory capabilities and invade neighboring tissues by degrading and moving through the ECM, and ultimately transit to secondary sites by finding their way into the blood and lymphatic circulation. Importantly, Ca\textsuperscript{2+} signaling plays a key role at a number of points in the invasion-metastatic cascade.

**Adhesion and Epithelial–Mesenchymal Transition (EMT)**

The invasion-metastatic cascade begins with the loss of cell–ECM and cell–cell adhesion. Cells are linked to the ECM at focal adhesion points by structural complexes connecting membrane spanning integrins to the cytoskeleton. And so, the rate of focal adhesion assembly and disassembly governs the cell’s migratory ability. The process of disassembly is Ca\textsuperscript{2+} sensitive and triggered by Ca\textsuperscript{2+} oscillations that promote the association of focal adhesion kinase (FAK), a regulator of focal adhesion turnover, with the focal adhesion complex (18). The Ca\textsuperscript{2+} oscillations, which are spatially restricted at the focal adhesions (19), increase the residency of FAK at these sites through Ca\textsuperscript{2+}/calmodulin-dependent protein kinase II (CaMKII)-dependent regulation of its phosphorylation status (19–22).

The loss of cell–cell adhesion is also mediated through the process of EMT (23). The EMT process converts polarized epithelial cells into highly motile mesenchymal cells, defined by the induction of the mesenchymal markers N-cadherin, vimentin, and transcription factors, Snail, Slug, and Twist. Induction of EMT in the MDA-MB-231 breast cancer cell line was dependent on increased store-operated Ca\textsuperscript{2+} entry (SOCE) driven by expression of the SOCE proteins, stromal interaction molecule 1 (STIM1) and Orai1 (24). The SOCE pathway is activated in response to depletion of ER Ca\textsuperscript{2+} stores, which causes the ER-localized STIM to bind to and open the plasma membrane Ca\textsuperscript{2+} channel Orai1 (25). In contrast, SOCE was not important for EMT in MDA-MB-468 breast cancer cells (26). In these cells, the Ca\textsuperscript{2+} permeable transient receptor potential canonical type 1 channel was implicated as a sensitizer to EMT (26). Moreover, a subsequent study of MDA-MB-468 cells showed a requirement for Ca\textsuperscript{2+}-permeable transient receptor potential melastatin-like 7 (TRPM7) channels in increasing vimentin expression through the signal transducer and activator of transcription 3 pathway (27). Collectively, these studies define a role for Ca\textsuperscript{2+} signaling in EMT and hint that EMT regulation by Ca\textsuperscript{2+} is likely to involve diverse mechanisms that are highly dependent on cell type and stimulus.

**Migration and ECM Degradation**

In the second step of the invasion-metastatic cascade, freely migrating cancer cells invade the surrounding stromal tissue. Migrating cells move by a cyclical process that begins with the extension of leading edge protrusions, known as lamellipodia. Lamellipodia attach to the substratum and contraction of the trailing rear edge moves the cell toward the lamellipodia (28, 29). At the leading edge, local Ca\textsuperscript{2+} signals control forward movement by regulating lamellipodia retraction and adhesion cycling through the activation of actin filament contraction (30, 31). Actin dynamics can also be influenced more indirectly by Ca\textsuperscript{2+} signaling, through activation of Ca\textsuperscript{2+}-dependent kinases (30, 32) and regulation of Rac1, RhoA, and Cdc42 GTPases (33–36). In addition to forward motion, directional steering is also dependent on spatially restricted Ca\textsuperscript{2+} signals. These events, termed “Ca\textsuperscript{2+} flickers,” are triggered by Ca\textsuperscript{2+} influx through TRPM7 channels and amplified by ER Ca\textsuperscript{2+} release via inositol 1,4,5-trisphosphate receptor type 2 activation (37). While these spatially localized Ca\textsuperscript{2+} influx play important roles at the leading edge, there is on average, a front-to-rear increase in Ca\textsuperscript{2+} concentration (38–40). At the trailing edge, Ca\textsuperscript{2+} signaling is determined, in large part, by Ca\textsuperscript{2+} influx through L-type Ca\textsuperscript{2+} channels, which serves to maintain contractility and stabilize directional movement (40).

Invasive cancer cells migrate through their surrounding tissue. They do this by proteolytically degrading the ECM with enzymes that include matrix metalloproteinases (MMPs) and cathepsins (41). Importantly, Ca\textsuperscript{2+} influx determines how these enzymes influence metastasis. In a prostate cancer cell study, the metastatic potential was dependent on the expression of MMP2, MMP9, and cathepsin B regulated by Ca\textsuperscript{2+} influx through the transient receptor potential melastatin 2 (TRPV2) channel (42). Another study identified a role for SOCE (43). Activation of STIM/Orai was shown to influence melanoma metastasis by maintaining levels of membrane type1 MMP (MT1-MMP) at the plasma membrane (43).
IN VolVEMENT OF ER-MITOCHONDRIA L Ca2++ FLUX IN CANCER CELL INVASION AND METASTASIS

Cancer causes transcriptional and functional changes that often affect regulators of cytoplasmic Ca2+, including the TRP channels and components of STIM/Orai-mediated Ca2+ entry, for reviews see Ref. (2, 13, 14, 44). These changes are likely to have the greatest impact on the spatiotemporal profile of the cytoplasmic Ca2+ signal and affect the invasion-metastatic cascade by impinging on the cytoplasmically localized effectors outlined above (22, 45). As alluded to earlier, cancer is also associated with altered mitochondrial Ca2+ handling, brought about by changes in the expression profile of the mitochondrial Ca2+ uptake machinery, as well as oncoproteins and tumor suppressors at the MAM (46). Recent investigations using primary tumor models and cancer cell lines support the concept that the survival advantages of altered mitochondrial Ca2+ derive from effects on cellular metabolism (47) and apoptosis sensitivity (48–52). In the following section, we assess the evidence that altered ER-mitochondrial Ca2+ is also a determinant of increased invasive and metastatic potential (Figure 1).

Mitochondria-Associated Membrane

The ER-localized inositol 1,4,5-trisphosphate receptor (IP3R) and ryanodine receptor Ca2+ release channels deliver Ca2+ to the mitochondria (53–55). Structural elements, which include physical tethers linking both membranes (56, 57) and protein–protein interactions that bridge the ER release and mitochondrial uptake machinery (58, 59), facilitate the Ca2+ transfer. To get to the matrix, Ca2+ first moves across the outer mitochondrial membrane through the voltage-dependent anion channel (VDAC) (60–62). The VDAC is a porin channel and diffusion pathway for ions and metabolites (62). Despite its large pore size, VDAC can function as a highly regulated Ca2+ permeability (63, 64) that directly couples to IP3R-dependent Ca2+ release through interactions with the mitochondrial chaperone GRP75 (58). From the intermembrane space, Ca2+ moves across the inner membrane through the mitochondrial Ca2+ uniporter (MCU) (65, 66). Cancer remodels the MAM architecture by changing the expression levels of ER and mitochondrial Ca2+ channel proteins or their associated binding partners and regulators (46). Restructuring the MAMs and the resultant effects on mitochondrial Ca2+ homeostasis impinges on many processes including metabolism, bioenergetics, cell death, proliferation, mitochondrial dynamics, and cytoplasmic Ca2+ signaling. For the purpose of this review, we restrict our focus to those effects that most profoundly impact, or are likely to impact, invasion and metastasis.

Voltage-Dependent Anion Channel

Expression of the VDAC1 isoform is robustly increased in many cancer cell types (67) and reliably predicts survival outcomes in breast, colon, and lung cancers (68, 69). Increased VDAC likely promotes cancer cell growth primarily by influencing mitochondrial metabolism and apoptosis (70–72), processes that are also tightly regulated by VDAC interactions with hexokinase and members of the Bcl-2 family (72–74). Importantly, VDAC1 knockdown reduced cancer cell migration in vitro and suppressed tumor growth in vivo (75, 76). A decrease in VDAC expression would be expected to limit mitochondrial Ca2+ uptake (61). Indeed, VDAC influences cell migration by a mechanism that involves the regulation of mitochondrial Ca2+ uptake by interactions with Bcl-2 family proteins. The structural determinants and functional correlates of the VDAC-Bcl-2 protein interactions have been well characterized, as reviewed in Ref. 67. Antiapoptotic Bcl-XL and MCL1 both bind to VDAC1 and VDAC3 isoforms...
to promote mitochondrial Ca\(^{2+}\) uptake and drive cell migration (77–80). Importantly, disrupting the Bcl-XL-VDAC and MCL1-VDAC interactions was found to inhibit migration of triple negative breast cancer cells (80) and non-small cell lung carcinoma cells, respectively (78). These data raise the possibility of suppressing invasion and metastasis by targeting VDAC-Bcl-2 protein interactions.

**Inositol 1,4,5-Trisphosphate Receptors**
The type 3 IP\(_3\)R isoform (IP\(_3\)R-3), which is absent in normal colorectal mucosa, is expressed in colorectal carcinoma. Moreover, the expression is greatest at the invasive margin and strongly correlated with metastasis and patient survival (81). The IP\(_3\)R-3 is also overexpressed in human glioblastoma tissue (82). Inhibiting IP\(_3\)R\(_s\) in glioblastoma cell lines reduced invasion in vitro; it also reduced invasion in vivo and prolonged survival by suppressing tumor growth (82). These studies, however, did not define the mechanisms by which increased IP\(_3\)R-3 expression directs invasion and metastasis. Interestingly, the IP\(_3\)R-3 is enriched in at the MAM in some cell types (83), and it may preferentially deliver Ca\(^{2+}\) to the mitochondria under certain conditions (84). It is possible then that increased IP\(_3\)R abundance promotes invasion and metastasis by increasing Ca\(^{2+}\) delivery to the mitochondria.

**Mitochondrial Ca\(^{2+}\) Uniporter**
The MCU machinery includes the MCU pore-forming subunit (65, 66) or its dominant-negative MCUb (85), together with associated regulators EMRE (86, 87) and MICU1-3 (88–91). Analysis of gene expression databases revealed that MCU levels are increased in several subtypes of breast cancer and correlated with tumor size, invasive and metastatic indices, and patient survival (92–94). While expression changes in the MCU regulators MCU1-3 and EMRE did not correlate with tumor size and invasiveness (92), poorer patient survival did correlate with increased MCU in combination with decreased MICU1 (94). The involvement of MCU in cancer progression was demonstrated in vivo by Tosatto et al., who showed that breast cancer tumor xenografts derived from MCU-deleted cells grew more slowly and were less likely to metastasize (92). In vitro experiments that knocked-down or inhibited MCU in breast cancer cell lines decreased mitochondrial Ca\(^{2+}\) uptake to inhibit migration and invasive potential without affecting cell survival (92, 94), proliferation, or apoptosis (95).

**MECHANISMS OF MITOCHONDRIAL Ca\(^{2+}\)-REGULATED INVASION AND METASTASIS**

**Store-Operated Ca\(^{2+}\) Entry (SOCE)**
Subplasmalemmal mitochondria regulate the activation and inactivation properties of SOCE by buffering incoming Ca\(^{2+}\) (96–98). Activation of SOCE is dependent on ER Ca\(^{2+}\) depletion, suggesting that ER, SOCE, and mitochondria are functionally coupled. Indeed, by limiting Ca\(^{2+}\) accumulation around the mouth of the IP\(_3\)R, mitochondrial Ca\(^{2+}\) uptake prevents Ca\(^{2+}\)-dependent inactivation of IP\(_3\)R\(_s\), which further depletes ER Ca\(^{2+}\) stores to promote SOCE (99). Given the Ca\(^{2+}\) communication between ER, mitochondrial and SOCE pathways, it is not surprising that MCU knockdown in MDA-MB-231 (93) and Hs578t (95) breast cancer lines inhibited both mitochondrial Ca\(^{2+}\) accumulation and SOCE. In the Hs578t cells, this caused a loss of cell polarity and migration associated with decreased RhoA, Rac1, and calpain activities (95). In these experiments, inhibiting SOCE (93, 95) or chelating intracellular Ca\(^{2+}\) (95) recapitulated the effects of MCU knockdown on migration. These data are consistent with studies defining STIM and Orai as key players in regulating invasion and metastasis (43, 100, 101) and suggest that altered MCU expression in cancer cells can influence downstream motility effectors by regulating SOCE.

**Mitochondrial Dynamics**
Mitochondria redistribute to the leading edge of cancer cells to support the increased bioenergetic demands at the invadopodia (102–105). Interestingly, the translocation of mitochondria to subplasmalemmal sites also plays a critical role during immune cell activation, where they regulate Ca\(^{2+}\) influx through SOCE (106). It is yet to be determined, however, if mitochondrial positioning, and its influence on Ca\(^{2+}\) influx, affects the polarization of cytoplasmic Ca\(^{2+}\) signaling in migrating cancer cells.

The translocation of mitochondria is dependent on increased mitochondrial fission, a process also promoted by mitochondrial Ca\(^{2+}\) accumulation (107, 108). Evidence that MCU plays a role in fission comes from the observation that fission is inhibited by the pharmacological block of the MCU (109, 110) and enhanced by loss-of-function mutations in MICU1, which promote mitochondrial Ca\(^{2+}\) uptake (111). Mechanistically, mitochondrial Ca\(^{2+}\) might influence fission by regulating the activity of dynamin-related protein 1 (Drp1). The ability of Drp1 to promote fission is dependent on phosphorylation at serine 616 (S616) and dephosphorylation of serine 637 (S637) (112, 113). Cytoplasmic Ca\(^{2+}\) signaling is known to regulate the phosphorylation status of Drp1 through calcineurin-dependent dephosphorylation of S637 (S637) (112, 113). Cytoplasmic Ca\(^{2+}\) signaling is known to regulate the phosphorylation status of Drp1 through calcineurin-dependent dephosphorylation of S637 (S637) (112, 114), and more recently it was found that blocking the MCU suppressed fission by decreasing Drp1 phosphorylation at S616 (115). These observations are relevant to this review because Drp1 is widely associated with tumor invasion and metastatic potential (104, 116–118), and increased S616 is found in breast cancer and lymph node metastases (104). Although speculative, it is possible that increased mitochondrial Ca\(^{2+}\) uptake in cancer cells links to invasion and metastasis through the processes of fission and mitochondrial localization.

**Bioenergetics**
Mitochondrial Ca\(^{2+}\) activates several Ca\(^{2+}\)-dependent enzymes involved in the tricarboxylic acid (TCA) cycle (119). Work by Cárdenas et al. showed that constitutive low-level ER-mitochondrial Ca\(^{2+}\) transfer maintains flux through the TCA cycle to fuel oxidative phosphorylation and ATP production (120). In a follow-up study, the same group showed that blocking IP\(_3\)R\(_s\) in cancer cells impaired oxidative phosphorylation, which killed cells by necrosis and reduced tumor growth in vivo (47). Although not examined in these studies, one might expect a similarly invoked bioenergetic...
crisis to inhibit cancer cell invasion and metastasis. Such an outcome is predicated based on the requirement for functional oxidative phosphorylation in tumor metastasis (121, 122), as well as the spatially restricted bioenergetic demands needed for cell migration (102–105).

**Reactive Oxygen Species (ROS)**

Mitochondrial ROS are generated as a consequence of normal respiration. As electrons supplied by the TCA cycle are passed down the electron transport chain, they escape, mostly at complex I and III, to react with O2 and produce ROS. Mitochondrial Ca2+ uptake can increase ROS production at complexes I, III and IV under a variety of conditions. Although the mechanisms are still unclear, a number of possibilities have been proposed, as reviewed previously (123, 124). Also, mitochondrial Ca2+ can promote the release of ROS accumulated in cristae and intermembrane spaces through Ca2+-dependent increases in matrix volume (125).

Increased mitochondrial ROS production is a known determinant of tumor growth and metastasis (126, 127) that likely drives invasion and metastasis by increasing cell migration (128–130). Importantly, increased migration is repeatedly correlated with increased ER-mitochondrial Ca2+ uptake (92–95). While excessive ROS production is toxic and excessive mitochondrial Ca2+ uptake inhibits rather than promotes migration (78, 131), physiological Ca2+-dependent ROS production is a major mitochondrially derived signal involved in regulating downstream effectors. In one study, increased ER-mitochondrial Ca2+ transfer in non-small cell lung carcinoma cells was caused by MCL1–VDAC interactions that promoted cell migration by increasing mitochondrial ROS production (78). In another study, increased mitochondrial Ca2+ uptake increased breast cancer cell xenograft growth and metastasis by increased ROS-dependent expression of HIF-1α (92). To our knowledge, HIF-1α is the only cell migration regulator that has been specifically linked to mitochondrial Ca2+-dependent ROS production. Nevertheless, many migration effectors are sensitive to ROS signaling (132). Perhaps more intriguingly, many of these, including the Rho GTPases, FAK, MMPs, and mediators of EMT, are sensitive to both ROS and Ca2+ signals, see Ref. (13, 132) for complete listings of ROS and Ca2+-sensitive targets, respectively. The degree of overlap between ROS and Ca2+-sensitive effectors highlights a need to carefully differentiate between ROS and Ca2+-dependent effects when probing the role of ER-mitochondrial Ca2+ transfer.

**CONCLUSION**

The molecular identification of the SOCE and MCU machinery, the introduction of powerful molecular tools, and the evolution of cancer genetics have all contributed to developing our understanding of how Ca2+ signals regulate cancer cell invasion and metastasis. As we have seen, a picture has emerged in which Ca2+ release, mitochondrial Ca2+ uptake, and plasmalemmal Ca2+ influx work together to exquisitely regulate cell motility. This complexity, however, should not dissuade efforts to examine the possibility of therapeutically targeting ER-mitochondrial Ca2+ transfer to affect metastasis. Encouragingly, many of the studies reviewed here have already demonstrated the feasibility of such an approach, showing reduced metastasis in vivo after targeting IP3Rs (47), STIM/Orai (22, 45), or MCU (92). In addition, therapeutics originally designed to promote cell death might also be useful for limiting metastasis. In this case, the Bcl-2 inhibitors, both BH3 and BH4 mimetics (80, 133), as well as recently developed MCL1 inhibitors (134), would be expected to suppress cell migration by limiting ER-mitochondrial Ca2+ transfer.

**AUTHOR CONTRIBUTIONS**

CW wrote the manuscript and designed and prepared figures.

**FUNDING**

Support was provided by Rosalind Franklin University of Medicine and Science.

**REFERENCES**

1. Clapham DE. Calcium signaling. Cell (2007) 131:1047–58. doi:10.1016/j.cell.2007.11.028
2. Hoth M. CRAC channels, calcium, and cancer in light of the driver and passenger concept. Biochim Biophys Acta (2016) 1863:1408–17. doi:10.1016/j.bbcan.2015.12.009
3. Stewart TA, Yapa KTDS, Monteith GR. Altered calcium signaling in cancer cells. Biochim Biophys Acta (2015) 1848:2502–11. doi:10.1016/j.bbcan.2014.08.016
4. Kandoth C, McClellan MD, Vandin F, Ye K, Niu B, Lu C, et al. Mutational landscape and significance across 12 major cancer types. Nature (2013) 502:333–9. doi:10.1038/nature12634
5. Tamborero D, Gonzalez-Perez A, Perez-Llamas C, Deu-Pons J, Kandoth C, Reimand J, et al. Comprehensive identification of mutational Cancer driver genes across 12 tumor types. Sci Rep (2013) 3:2650. doi:10.1038/srep02650
6. Marchi S, Pinton P. Alterations of calcium homeostasis in cancer cells. Curr Opin Pharmacol (2016) 29:1–6. doi:10.1016/j.coph.2016.03.002
7. Verfliet T, Parys JB, Bültyneck G. Bcl-2 proteins and calcium signaling: complexity beneath the surface. Oncogene (2016) 35:5079–92. doi:10.1038/onc.2016.31
8. Sung PJ, Tsai FD, Vais H, Court H, Yang J, Fehrenbacher N, et al. Phosphorylated K-Ras limits cell survival by blocking Bcl-xL sensitization of inositol trisphosphate receptors. Proc Natl Acad Sci U S A (2013) 110:20593–8. doi:10.1073/pnas.1306431110
9. Rimesi A, Marchi S, Paterniani S, Pinton P. H-Ras-driven tumoral maintenance is sustained through caveolin-1-dependent alterations in calcium signaling. Oncogene (2014) 33:3239–40. doi:10.1038/onc.2013.192
10. Giorgi C, Ito K, Lin H-K, Santangelo C, Wieckowski MR, Lebiedzinska M, et al. p53 at the endoplasmic reticulum regulates apoptosis in a Ca2+-dependent manner. Nat Rev Cancer (2011) 11:609–18. doi:10.1038/nrc3105
11. Giorgi C, Bonora M, Sorrentino G, Missiroli S, Poletti F, Suski JM, et al. PML regulates apoptosis at endoplasmic reticulum by modulating calcium release. Science (2010) 330:1247–51. doi:10.1126/science.1189157
12. Hedgepeth SC, Garcia MI, Wagner LE II, Rodriguez AM, Chintapalli SV, Snyder RR, et al. The BRCA1 tumor suppressor binds to inositol 1,4,5-trisphosphate receptors to stimulate apoptotic calcium release. J Biol Chem (2015) 290:7304–13. doi:10.1074/jbc.M114.611186
13. Prevorskaya N, Skryma R, Shuba Y. Calcium in tumour metastasis: new roles for known actors. Nat Rev Cancer (2011) 11:609–18. doi:10.1038/nrc3105
14. Tsai F-C, Kuo G-H, Chang S-W, Tsai P-J. Ca2+ signaling in cytoskeletal reorganization, cell migration, and cancer metastasis. Biomed Res Int (2015) 2015:409245. doi:10.1155/2015/409245
15. Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. Science (2011) 331:1559–64. doi:10.1126/science.1205543

16. Talmadge JE, Fidler IJ. AACR centennial series: the biology of cancer metastasis: historical perspective. Cancer Res (2010) 70:5649–69. doi:10.1158/0008-5472.CAN-10-1040

17. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell (2011) 144:64–74. doi:10.1016/j.cell.2011.02.013

18. Giannone G, Rondé P, Gaire M, Beaudouin J, Haiech J, Ellenberg J, et al. Calcium oscillations promote neuronal motility. Neuron (2006) 9:50–7. doi:10.1016/j.neuron.200512.023

19. Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. Nature (2010) 469:861–73. doi:10.1038/nature09465

20. Price JT, Tiganis T, Agarwal A, Djakiew D, Thompson EW. Epidermal growth factor receptor stimulation elicits translocation and activation of Rac. J Biol Chem (2003) 278:39413–21. doi:10.1074/jbc.M302083200

21. Kesselbrock K, Plaks V, Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. Cell (2010) 141:52–67. doi:10.1016/j.cell.2010.03.015

22. Chen Y-F, Chiu W-T, Chen Y-T, Lin P-Y, Huang H-J, Chou C-Y, et al. Calcium signal dependent. Biochim Biophys Acta (2016) 1864(6):858–64. doi:10.1016/j.bbamcr.2016.12.024

23. Thiery JP, Acloque H, Huang RYJ, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell (2009) 139:871–90. doi:10.1016/j.cell.2009.11.007

24. Hu J, Qin K, Zhang Y, Gong J, Li N, Lv D, et al. Downregulation of transcription factor Oct4 induces an epithelial-to-mesenchymal transition via enhancement of Ca2+ influx in breast cancer cells. Biochem Biophys Res Commun (2011) 411:786–91. doi:10.1016/j.bbrc.2011.07.025

25. Petrie RJ, Doyle AD, Yamada KM. Random versus directionally persistent cell migration. J Cell Sci (2016) 129:6203. doi:10.1242/jcs.196432

26. Davis FM, Peters AA, Grice DM, Cabot PJ, Parat M-O, Roberts-Thomson SJ, et al. Calcium oscillations locally trigger focal adhesion disassembly and enhance residency of focal adhesion kinase at focal adhesions. J Biol Chem (2004) 279:28715–23. doi:10.1074/jbc.M400540200

27. Wei C, Wang X, Chen M, Ouyang K, Song L-S, Cheng H. Calcium flickers steer cell migration. Nature (2009) 457:901–5. doi:10.1038/nature07577

28. Missiroli S, Danese A, Iannitti T, Paternaghi S, Perrone M, Previati M, et al. Endoplasmic reticulum–mitochondria Ca2+ crosstalk in the control of the tumor cell fate. Biochim Biophys Acta (2017) 1866(6):858–64. doi:10.1016/j.bbamcr.2016.12.024

29. Cárdenas C, Müller M, McNeal A, Lovy A, Jaya F, Bustos G, et al. Selective vulnerability of cancer cells by inhibition of Ca2+ transfer from endoplasmic reticulum to mitochondria. Cell (2016) 164:23–24. doi:10.1016/j.cell.2016.02.030

30. Monet M, Lehen’kyi V, Gackiere F, Firlej V, Vandenberghe M, Roudbaraki M, et al. Intravital imaging reveals p53-dependent cancer cell death induced by phototherapy via calcium signaling. Oncotarget (2015) 6:1345–45. doi:10.18632/oncotarget.9235

31.สวยกุมา M, Granatiero V, Sbiera S, Sbiera I, Lacas-Gervais S, et al. Role of cationic channel TRPV2 in promoting prostate cancer migration and progression to androgen resistance. Cancer Res (2010) 70:1225–35. doi:10.1158/0008-5472.CAN-09-2205

32. Motoiani RK, Zhang X, Harmon KE, Keller RS, Matrougui K, Bennett JA, et al. Orai1 is an estrogen receptor α-regulated Ca2+ channel that promotes tumorigenesis. FASEB J (2013) 27:63–75. doi:10.1096/fj.12-213801

33. Evans JH, Falke JJ. Ca2+ oscillations and mitochondria. J Biomed Sci (2014) 21:140. doi:10.1186/1340-2594-21-140

34. Monet M, Lehen’kyi V, Gackiere F, Firlej V, Vandenberghe M, Roudbaraki M, et al. Calcium oscillations locally trigger focal adhesion disassembly and enhance residency of focal adhesion kinase at focal adhesions. J Biol Chem (2004) 279:28715–23. doi:10.1074/jbc.M400540200

35. Davis FM, Azimi I, Faville RA, Peters AA, Jalink K, Putney JW, et al. Induction of calcium oscillations trigger focal adhesion disassembly in human U87 astrocytoma cells. J Biol Chem (2002) 277:26364–71. doi:10.1074/jbc.M203952200

36. Rizzuto R, Pinton P, Carrington W, Fay FS, Fogarty KE, Lifshitz LM, et al. Close association of mitochondrial Ca2+ stores with the endoplasmic reticulum. Biochim Biophys Acta (2017) 1864(6):858–64. doi:10.1016/j.bbamcr.2016.12.024

37. Doghman-Bouguerra M, Granatiiero V, Sbiera S, Sbiera I, Lacas-Gervais S, et al. Calcium oscillations locally trigger focal adhesion disassembly and enhance residency of focal adhesion kinase at focal adhesions. J Biol Chem (2004) 279:28715–23. doi:10.1074/jbc.M400540200

38. Csordás G, Renken C, Várnai P, Walter L, Weaver D, Buttle KF, et al. Structural and functional significance of the physical linkage between ER and mitochondria. J Cell Biol (2007) 177:373–84. doi:10.1083/jcb.20060416
and mitochondrial Ca\textsuperscript{2+} channels. *J Cell Biol* (2006) 175:901–11. doi:10.1083/jcb.200608073

59. Bi CC, Stracka D, Prescianotto-Baschong C, Frieden M, Demaurex N, Hall MN. Feature article: mTOR complex 2-Akt signaling at mitochondria-associated endoplasmic reticulum membranes (MAM) regulates mitochondrial physiology. *Proc Natl Acad Sci U S A* (2013) 110:12526–34. doi:10.1073/pnas.1302451110

60. De Stefani D, Bonomi A, Romagnoli A, Messina A, De Pinto V, Pinton P, et al. VDAC1 selectively transfers apoptotic Ca\textsuperscript{2+} signals to mitochondria. *Cell Death Differ* (2012) 19(2):267–73. doi:10.1038/cdd.2011.92

61. Rapizzi E, Pinton P, Szabadkai G, Wieckowski MR, Vandreastele G, Baird G, et al. Reconstituent expression of the voltage-dependent anion channel enhances the transfer of Ca\textsuperscript{2+} microdomains to mitochondria. *J Cell Biol* (2002) 159:613–24. doi:10.1083/jcb.200205091

62. Colombini M. VDAC structure, selectivity, and dynamics. *Biochim Biophys Acta* (2012) 1818:1457–65. doi:10.1016/j.bbamem.2011.12.026

63. Bathori G, Csordas G, Garcia-Perez C, Davies E, Hajnoczky G. Ca\textsuperscript{2+}–dependent control of the permeability properties of the mitochondrial outer membrane and voltage-dependent anion-selective channel (VDAC).* J Biol Chem* (2006) 281:17347–58. doi:10.1074/jbc.M60906200

64. Tan W, Colombini M. VDAC closure increases calcium ion flux. *Biochim Biophys Acta* (2007) 1768:2510–5. doi:10.1016/j.bbamem.2007.06.002

65. Baughman JM, Perocchi F, Girgis HS, Plovanich M, Belcher-Timme CA, Tan W, Colombini M. VDAC closure increases calcium ion flux. *Biophys Acta* (2006) 159:613–24. doi:10.1083/jcb.200205091

66. De Stefani D, Raffaello A, Teardo E, Zerbi A, Pinton P, Colombini M. Voltage-dependent anion channel-1 associated genes predicts recurrence-free survival of colorectal carcinoma. *Oncotarget* (2015) 6:1482. doi:10.1038/onctarget.2014.419

67. Shoshan-Barmatz V, Ben-Hail D, Admoni L, Krelin Y, Tripathi SS. The mitochondrial calcium uniporter. *Proc Natl Acad Sci U S A* (2010) 107:12526–34. doi:10.1073/pnas.1006623107

68. Ko J-H, Gu W, Lim I, Zhou T, Bang H. Expression profiling of mitochondrial voltage-dependent anion channel (VDAC) in uterine cervical cancer and its action on cervical cancer cells. *Oncotarget* (2017) 8:22649–59. doi:10.18632/oncotarget.17347

69. Shimizu S, Matsuoka Y, Shinohara Y, Oneda Y, Tsujimoto Y. Essential role of voltage-dependent anion channel-1 in uterine cervical cancer and its association with mitochondrial calcium ion transport. *J Biol Chem* (2015) 290:341–5. doi:10.1074/jbc.M114.584530

70. Vais H, Mallilankaraman K, Mak D-OD, Hoff H, Payne R, Tanis JE, et al. Recombinant expression of the voltage-dependent anion channel (VDAC) promotes mitochondrial Ca\textsuperscript{2+} uptake and reactive oxygen species generation. *Cell Death Dis* (2014) 5:e1482. doi:10.1038/cddis.2014.419

71. Buvat C, Sancak Y, et al. Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter. *Nature* (2011) 476:341–5. doi:10.1038/nature10230

72. Rajan S, Choi M, Nguyen QT, Ye H, Liu W, Toh HT, et al. Structural transition of mitochondrial voltage-dependent anion channel-1 associated with aggressiveness of colorectal carcinoma. *Cell Calcium* (2010) 48:313–23. doi:10.1016/j.cca.2010.09.005

73. Tosatto A, Sommaggio R, Kummerow C, Bentham RB, Blacker TS, Berecz T, et al. The mitochondrial calcium uniporter subtype 3 blocks glioblastoma invasion and extends survival. *Cancer Res* (2015) 75:1173–83. doi:10.1158/0008-5472.CAN-14-2988

74. Hall DD, Wu Y, Domann FE, Spitz DR, Anderson ME. Mitochondrial calcium uniporter gating: a mechanism for sustained signaling and transcriptional activation in T lymphocytes. *Proc Natl Acad Sci U S A* (2007) 104:13024–5. doi:10.1073/pnas.0702452104

75. Fouqué A, Lepvrier E, Debure L, Gouriou Y, Malleter M, Delcroix V, et al. MICU1 and MICU2 finely tune the mitochondrial Ca\textsuperscript{2+} uniporter activity is dispensable for MDA-MB-231 breast carcinoma cell migration. *Cell Death Differ* (2014) 21:458–66. doi:10.1038/cdd.2013.157

76. Mendes CCP, Gomes DA, Thompson M, Souto NC, Goes AM, et al. The type III inositol 1,4,5-trisphosphate receptor preferentially transmits apoptotic Ca\textsuperscript{2+} signals into mitochondria. *J Biol Chem* (2005) 280:40892–900. doi:10.1074/jbc.M506623200

77. Plovanich M, Bogorad RL, Sancak Y, Kamer KJ, Strittmatter L, Li AA, et al. MICU1 is a essential gatekeeper for MCU-mediated mitochondrial Ca\textsuperscript{2+} uptake that regulates cell survival. *Cell* (2012) 151:630–44. doi:10.1016/j.cell.2012.10.011

78. Perocchi F, Gohil VM, Girgis HS, Bao XR, McCombs JE, Palmer AE, et al. MICU1 encodes a mitochondrial EF hand protein required for Ca\textsuperscript{2+} uptake. *Nature* (2010) 467:291–6. doi:10.1038/nature09338

79. Prudent J, Popgeorgiev N, Gadet R, Deygas M, Rimokh R, Gillet G. Mitochondrial subtype 3 blocks glioblastoma invasion and extends survival. *Oncotarget* (2015) 6:36570. doi:10.18632/oncotarget.6704

80. Tosatto A, Sommaggio R, Kummerow C, Bentham RB, Blacker TS, Berecz T, et al. The mitochondrial calcium uniporter regulates breast cancer progression via HIF-1\alpha. *EMBO Mol Med* (2016) 8:369–85. doi:10.15252/emmm.201606255

81. Tang S, Wang X, Shen Q, Yang X, Yu C, Cai C, et al. Mitochondrial Ca\textsuperscript{2+} uptake controls actin cytoskeleton dynamics during cell migration. *Biophys J* (2014) 107:726–37. doi:10.1016/j.bpj.2014.01.013

82. Haghighatgil AR, Sahebkar A, Respiratory mitochondria determine the pattern of activation and inactivation of the store-operated Ca\textsuperscript{2+} current (ICRAC). *EMBO J* (2000) 19:6401–7. doi:10.1093/emboj/19.23.6401
98. Hoth M, Fanger CM, Lewis RS. Mitochondrial regulation of store-operated calcium signaling in T lymphocytes. *J Cell Biol* (1997) 137:633–48. doi:10.1083/jcb.137.3.633

99. Deak AT, Blass S, Khan MJ, Groschner LN, Waldeck-Weiermair M, Hallström S, et al. IP3-activated STIM1 oligomerization requires intact mitochondrial Ca2+ uptake. *J Cell Sci* (2014) 127:2944–55. doi:10.1242/jcs.149807

100. Yang S, Zhang JH, Huang X-Y. Orai1 and STIM1 are critical for breast tumor cell migration and metastasis. *Cancer Cell* (2009) 15:124–34. doi:10.1016/j.ccr.2008.12.019

101. Yang N, Tang Y, Wang F, Zhang H, Xu D, Shen Y, et al. Blockade of store-operated Ca(2+) entry inhibits hepatocarcinoma cell invasion and migration by regulating focal adhesion turnover. *Cancer Lett* (2013) 330:163–9. doi:10.1016/j.canlet.2012.11.040

102. Desai SP, Bhatia SN, Toner M, Irimia D. Mitochondrial localization and the persistent migration of epithelial cancer cells. *Biophys J* (2013) 104:2077–88. doi:10.1016/j.bpj.2013.03.025

103. Jung J-U, Ravi S, Lee DW, McFadden K, Kamradt ML, Toussaint LG, et al. NIK/MAP3K14 regulates mitochondrial dynamics and trafficking to promote cell invasion. *Curr Biol* (2016) 26:3288–302. doi:10.1016/j.cub.2016.10.009

104. Zhao J, Zhang J, Yu M, Xie Y, Huang Y, Wolff DW, et al. Mitochondrial dynamics regulates migration and invasion of breast cancer cells. *Oncogene* (2013) 32:4814–24. doi:10.1038/onc.2012.494

105. Caino MC, Altieri DC. Cancer cells exploit adaptive mitochondrial dynamics to increase tumour invasion. *Cell Cycle* (2015) 14:3422–7. doi:10.1080/15384101.2015.1084448

106. Schwindling C, Quintana A, Krause E, Hoth M. Mitochondria positioning and cell death. *EMBO Rep* (2016) 17:1157–68. doi:10.15252/embr.201640895

107. Yang S, Zhang JH, Huang X-Y. Orai1 and STIM1 are critical for breast tumor cell migration and metastasis. *Cancer Cell* (2009) 15:124–34. doi:10.1016/j.ccr.2008.12.019

108. Deak AT, Blass S, Khan MJ, Groschner LN, Waldeck-Weiermair M, Hallström S, et al. IP3-activated STIM1 oligomerization requires intact mitochondrial Ca2+ uptake. *J Cell Sci* (2014) 127:2944–55. doi:10.1242/jcs.149807

109. Yang S, Zhang JH, Huang X-Y. Orai1 and STIM1 are critical for breast tumor cell migration and metastasis. *Cancer Cell* (2009) 15:124–34. doi:10.1016/j.ccr.2008.12.019

110. Yang N, Tang Y, Wang F, Zhang H, Xu D, Shen Y, et al. Blockade of store-operated Ca(2+) entry inhibits hepatocarcinoma cell invasion and migration by regulating focal adhesion turnover. *Cancer Lett* (2013) 330:163–9. doi:10.1016/j.canlet.2012.11.040

111. Desai SP, Bhatia SN, Toner M, Irimia D. Mitochondrial localization and the persistent migration of epithelial cancer cells. *Biophys J* (2013) 104:2077–88. doi:10.1016/j.bpj.2013.03.025

112. Jung J-U, Ravi S, Lee DW, McFadden K, Kamradt ML, Toussaint LG, et al. NIK/MAP3K14 regulates mitochondrial dynamics and trafficking to promote cell invasion. *Curr Biol* (2016) 26:3288–302. doi:10.1016/j.cub.2016.10.009

113. Zhao J, Zhang J, Yu M, Xie Y, Huang Y, Wolff DW, et al. Mitochondrial dynamics regulates migration and invasion of breast cancer cells. *Oncogene* (2013) 32:4814–24. doi:10.1038/onc.2012.494

114. Caino MC, Altieri DC. Cancer cells exploit adaptive mitochondrial dynamics to increase tumour invasion. *Cell Cycle* (2015) 14:3422–7. doi:10.1080/15384101.2015.1084448

115. Schwindling C, Quintana A, Krause E, Hoth M. Mitochondria positioning and cell death. *EMBO Rep* (2016) 17:1157–68. doi:10.15252/embr.201640895

116. Yang S, Zhang JH, Huang X-Y. Orai1 and STIM1 are critical for breast tumor cell migration and metastasis. *Cancer Cell* (2009) 15:124–34. doi:10.1016/j.ccr.2008.12.019

117. Deak AT, Blass S, Khan MJ, Groschner LN, Waldeck-Weiermair M, Hallström S, et al. IP3-activated STIM1 oligomerization requires intact mitochondrial Ca2+ uptake. *J Cell Sci* (2014) 127:2944–55. doi:10.1242/jcs.149807

118. Glancy R, Balaban RS. Role of mitochondrial Ca2+ in the regulation of cellular energetics. *Biochemistry* (2012) 51:2959–73. doi:10.1021/bi2018909

119. Cárdenas C, Miller RA, Smith I, Bui T, Molgó J, Pérez-Escuredo J, et al. Essential regulation of cell bioenergetics by constitutive InsP3 receptor Ca2+ transfer to mitochondria. *Cell* (2010) 142:270–83. doi:10.1016/j.cell.2010.06.007

120. Tochhawng L, Deng S, Pervaiz S, Yapp CT. Redox regulation of cancer cell invasion and migration. *EMBO Rep* (2016) 17:1157–68. doi:10.15252/embr.201640895

121. Tan AS, Baty JW, Dong L-F, Bezawork-Geleta A, Endaya B, Goodwin J, et al. Mitochondrial genome acquisition restores respiratory function and oxidative phosphorylation in cancer cells to promote metastasis. *Nat Cell Biol* (2014) 16:992–1003. doi:10.1038/ncl3639

122. Prokkes PS, Yoon Y, Robotam JL, Anders MW, Sheu S-S, Calcium, ATP, and ROS: a mitochondrial love-hate triangle. *Am J Physiol Cell Physiol* (2004) 287:C817–33. doi:10.1152/ajpcell.00139.2004

123. Tochhawng L, Deng S, Pervaiz S, Yapp CT. Redox regulation of cancer cell invasion and migration. *EMBO Rep* (2016) 17:1157–68. doi:10.15252/embr.201640895

124. Zorov DB, Juhászova M, Sollitt SJ. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol Rev* (2014) 94:9490–50. doi:10.1152/physrev.00006.2013

125. Booth DM, Enyedi B, Geiszt M, Várnai P, Hajnóczky G. Redox nanodomains are induced by and control singlet oxygen signaling at the ER-mitochondrial interface. *Mol Cell* (2016) 63:240–8. doi:10.1016/j.molcel.2016.05.040

126. Pelicano H, Lu W, Zhou Y, Zhang W, Chen Z, Hu Y, et al. Mitochondrial dysfunction and reactive oxygen species imbalance promote breast cancer cell motility through a CXCL14-mediated mechanism. *Cancer Res* (2009) 69:2375–83. doi:10.1158/0008-5427.CAN-08-3359

127. LeBlus VS, O’Connell JT, Gonzalez Herrera KN, Wikman H, Pantel K, Haigis MC, et al. PGC-1α mediates mitochondrial biogenesis and oxidative phosphorylation in cancer cells to promote metastasis. *Nat Cell Biol* (2014) 16:992–1003. doi:10.1038/ncl3639

128. LeBlus VS, O’Connell JT, Gonzalez Herrera KN, Wikman H, Pantel K, Haigis MC, et al. PGC-1α mediates mitochondrial biogenesis and oxidative phosphorylation in cancer cells to promote metastasis. *Nat Cell Biol* (2014) 16:992–1003. doi:10.1038/ncl3639

129. Kashatus JA, Nascimento A, Myers LJ, Sher A, Byrne FL, Hoehn KL, et al. Erk2 phosphorylation of Drp1 promotes mitochondrial fission and MAPK-driven tumor growth. *Mol Cell* (2015) 57:537–51. doi:10.1016/j.molcel.2015.01.002