Biochemical and Biophysical Research Communications 458 (2015) 186–193

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Mitochondrial Ca^{2+} uniporter is critical for store-operated Ca^{2+} entry-dependent breast cancer cell migration

Shihao Tang [a, d, 1], Xubu Wang [a, 1], Qiang Shen [b, 1], Xinyi Yang [a], Changhui Yu [a], Chunqing Cai [a], Guoshuai Cai [c], Xiaojing Meng [a, *], Fei Zou [a, *]

[a] Department of Occupational Health and Occupational Medicine, School of Public Health and Tropical Medicine, Southern Medical University, Guangzhou, Guangdong, China
[b] Department of Clinical Cancer Prevention, The University of Texas MD Anderson Cancer Center, Houston, TX, USA
[c] Department of Bioinformatics and Computational Biology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA
[d] Guangzhou No.12 Hospital, Guangzhou, China

ARTICLE INFO

Article history:
Received 11 January 2015
Available online 29 January 2015

Keywords:
MCU
Breast tumor
Migration
SOCE

ABSTRACT

Metastasis of cancer cells is a complicated multistep process requiring extensive and continuous cytosolic calcium modulation. Mitochondrial Ca^{2+} uniporter (MCU), a regulator of mitochondrial Ca^{2+} uptake, has been implicated in energy metabolism and various cellular signaling processes. However, whether MCU contributes to cancer cell migration has not been established. Here we examined the expression of MCU mRNA in the Oncomine database and found that MCU is correlated to metastasis and invasive breast cancer. MCU inhibition by ruthenium red (RuR) or MCU silencing by siRNA abolished serum-induced migration in MDA-MB-231 breast cancer cells and reduced serum- or thapsigargin (TG)-induced store-operated Ca2+ entry (SOCE). Serum-induced migrations in MDA-MB-231 cells were blocked by SOCE inhibitors. Our results demonstrate that MCU plays a critical role in breast cancer cell migration by regulating SOCE.

1. Introduction

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females, accounting for 23% of the total cancer cases and 14% of the cancer deaths worldwide [1]. Despite the significant improvement in both diagnostic and therapeutic modalities for breast cancer patients, metastasis still represents as the major cause of mortality. Therefore, a better understanding of molecular mechanisms involved in cancer metastasis will contribute to develop more effective and rational therapies for breast cancer.

Extracellular Ca^{2+} entry and subsequent elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) play a critical role in many aspects of cancer traits [2–4]. Previous researches have shown that Ca^{2+} influx is essential for migration of various cell types, including cancer cells [5,6]. Mitochondria are known not only to be a passive Ca^{2+} sink to store Ca^{2+} ions, but also regulates cellular Ca^{2+} homeostasis. Mitochondrial Ca^{2+} homeostasis plays a key role in the regulation of aerobic metabolism and cell survival [7]. Four categories of molecules have been identified to be related to mitochondrial Ca^{2+} influx mechanisms, including mitochondrial ryanodine receptor, mitochondrial uncoupling proteins, LETM1(Ca^{2+}/H^{+} exchanger), and Mitochondrial Ca^{2+} uniporter (MCU) [8]. MCU is a main Ca^{2+} channel existing in mitochondrial inner membrane, which is highly selective for Ca^{2+} and sensitive to a commonly used inhibitor, ruthenium red (RuR). Until recently, the molecular identity of MCU was just unveiled [9,10].

Increasing evidence has been showing the participation of mitochondria in the control of global cellular Ca^{2+} homeostasis by regulating the store-operated calcium entry (SOCE) [11–13]. In nonexcitable cells, SOCE is the predominant Ca^{2+} entry mechanism, which is activated when intracellular stores, such as endoplasmic reticulum (ER), release their stored Ca^{2+} [14]. ER Ca^{2+} stores could become depleted physiologically as a consequence of signaling mechanisms from activated G-protein-coupled or tyrosine kinase receptors [15]. Respiring mitochondria rapidly take up Ca^{2+} released from the stores, resulting in more extensive store depletion and thus robust activation of SOCE. Furthermore, by buffering

* Corresponding authors. Fax: +86 20 61648324.
E-mail addresses: xiaojingmeng@smu.edu.cn (X. Meng), zoufei616@163.com (F. Zou).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2015.01.092
0006-291X/

some of the incoming Ca^{2+}, mitochondria reduce Ca^{2+}-dependent inactivation of the calcium release-activated calcium (CRAC) channels, resulting in more prolonged Ca^{2+} influx [11]. Recently, SOCE was found to be critical for breast cancer cell migration and metastasis [5]. Although MCU is implicated in the regulation of SOCE and intracellular Ca^{2+} homeostasis, a role of MCU in the context of cancer cell migration has not been defined.

Here, we determined whether MCU expression is increased in metastatic human breast cancer and investigated the contribution of MCU in regulating breast cancer cell migration. MDA-MB-231 cells were employed in our research because they are more invasive than other breast cancer cells. By using MCU inhibitor RuR and down-regulating of the expression of MCU protein by specific siRNAs, we demonstrated that MCU inhibition and silencing abrogate serum-induced SOCE and cell migration in MDA-MB-231 cells. This is the first report demonstrating that MCU protein is critically involved in cancer cell migration via regulating SOCE.

2. Materials and methods

2.1. Reagents and antibodies

$GdCl_3$, $CaCl_2$, RuR, SKF96365 and 2-APB were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Roswell Park Memorial Institute (RPMI) 1640 culture medium, fetal bovine serum (FBS), Lipo2000 transfection Kit and endotoxin-free PBS (pH 7.4) without calcium and magnesium were acquired from Life Technologies (Carlsbad, CA, USA). Fluo-4 acetoxymethyl ester (Fluo-4/AM) and rhod-2—acetoxymethyl ester (rhod-2/AM) were purchased from Dojindo Laboratories (Kumamoto, Japan). All working solutions were prepared on the day of experiment. Rabbit polyclonal antibody to MCU and mouse polyclonal antibody to GAPDH were from Sigma and Abcam (Cambridge, MA, USA) respectively.

2.2. Oncomine data mining

We analyzed the gene expression datasets of the cancer tissue microarray database in Oncomine™ (Compendia Bioscience, Ann Arbor, MI, http://www.oncomine.org [16,17]. The Oncomine is a database bank in that gene expression profile data can be queried and analyzed for selected genes across the databases available to the public. These datasets provide fold-change values of gene expression and statistical significance as determined by P values, involving comparison between cancer and normal samples, and subtypes of breast cancer in relation to clinical-pathological significance of the patients. We extracted and compared MCU expression in normal vs. breast cancer tissues, ductal carcinoma in situ vs. invasive ductal breast cancer.

2.3. Cell culture and cell viability assays

MDA-MB-231 cells (ATCC® HTB-26™) were maintained in Dulbecco's modified Eagle's medium (DMEM) with penicillin G, streptomycin sulfate, 10% fetal bovine serum, and 4 mmol/L L-glutamine. Cell viability was evaluated by the MTT assay. The experiment was repeated three times.

2.4. $[Ca^{2+}]_c$ and $[Ca^{2+}]_{mt}$ measurements

Intracellular Ca^{2+} was monitored using Ca^{2+} sensitive fluorescent indicator, Fluo-4/AM by an inverted laser scanning confocal microscope (Olympus, FV1000-IX71, Japan). Cells were loaded in PBS with 2 μM Fluo-4/AM at 37 °C for 30 min in dark, rinsed twice with PBS and kept at room temperature for 20 min to allow de-esterification of Fluo-4 ester. Before experiment, Ca^{2+}-free PBS with 0.3 mM EGTA was added. Green fluorescence of Fluo-4 was excited by a 10 mW multi-tune argon laser at 488 nm, and emitted fluorescence was recorded through a 525 nm channel. For imaging using Fluo-4, $[Ca^{2+}]_i$ changes are presented as F/F_0 after background subtraction, where F is the change in fluorescence signal intensity and F_0 is the baseline as calculated by averaging 20 frames before stimulus application. To determine mitochondrial Ca^{2+} $[Ca^{2+}]_{mt}$, MDA-MB-231 cells were incubated with the cell-permeable Ca^{2+}-indicator rhod-2 AM (3 μmol/L; dissolved in DMSO) in DMEM for 1 h at 37 °C, and then washed for 0.5 h in rhod-2-free DMEM to allow de-esterification. To determine $[Ca^{2+}]_{mt}$, rhod-2 was excited at $\lambda exc = 540$ nm, and fluorescence was recorded at $\lambda em = 605$ nm [18].

2.5. In vitro wound-healing assay

Cells in medium containing 3% FBS were seeded into wells of 24-multiwell plates (Becton Dickinson). After the cells grew to confluence, wounds were made by sterile pipette tips. Cells were washed with PBS and refreshed with medium with or without 3% FBS. After 48 h incubation at 37 °C, the cells were fixed and photographed as described previously [19].

2.6. Transwell cell-migration assay

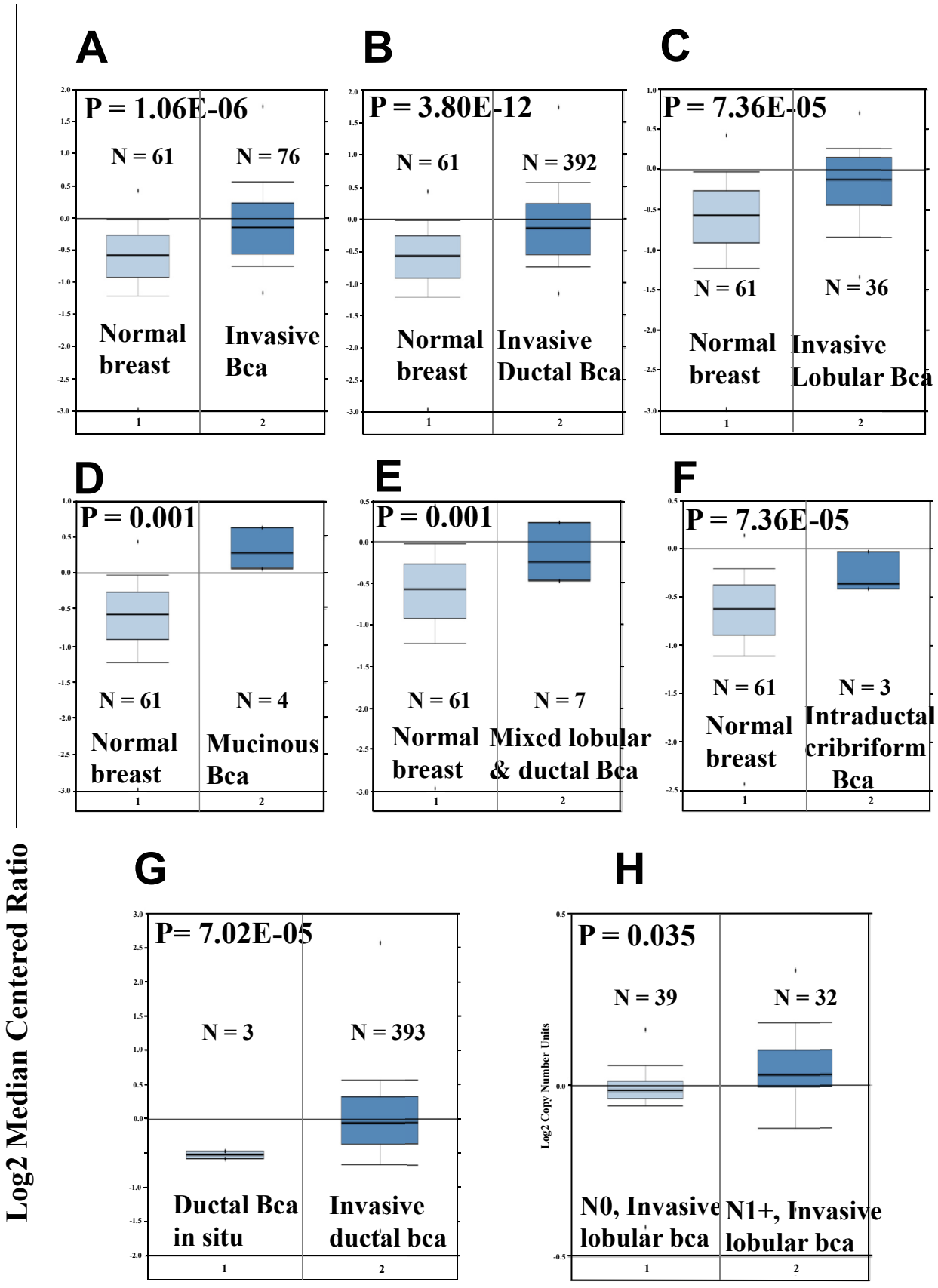
Transwell migration assays were performed as described previously [20]. The migration of MDA-MB-231 cells was assessed using 24-well transwell with an 8-μm pore size (Millipore). A total of 4×10^4 cells in serum-free DMEM (100 μl) were added to the upper chamber, and 500 μl of DMEM with 10% FBS were added to the lower chamber. Transwells were incubated for 18 h at 37 °C. Cells on the inside of the transwell inserts were removed with a cotton swab, and cells on the underside of the insert were fixed and stained. Photographs of three random fields were taken, and the cells were counted to calculate the average number of cells that had transmigrated.

2.7. Cell transfection

RNAi against MCU was performed in MDA-MB-231 cells using Lipo2000 transfection Kit. To silence MCU specific siRNA were designed: siRNA-MCU#1: nucleotides 899—917 of the corresponding mRNA (5′-GCCAGAGACAGACAAUACUtt-3′ and 3’-ttCGGUCUCUGUCUGUUAUGA-5’. siRNA-MCU#2: nucleotides 360—378 of the corresponding mRNA (5′-GGGAAUUGACAGAGUUGCUtt-3′ and 3’-ttCCCUUAACUGUCUCAACGA-5′). The non-targeting siRNA (scrambled) is the following: 5′-GCCUAAGAACGACAAAUCAtt-3′ and 3’-ttCGGAUUCUUGCUGUUUAGU-5’ [10]. These were transfected into cells in the absence of antibiotics using Lipofectamine 2000 according to the manufacturer's instructions. The scrambled siRNA were used as controls.

2.8. Western blotting

Cell lysates in RIPA buffer (50 μg of proteins in denaturing conditions) were subjected to SDS-PAGE (10%) and electro-transferred onto polyvinylidene fluoride membranes. After blocking with 5% nonfat dry milk dissolved in Tris-buffered saline containing 0.1% Tween 20 (TBST) for 2 h at room temperature, Western blots were probed overnight at 4 °C, with specific primary antibodies in TBST containing 5% BSA. The primary antibody used was rabbit polyclonal MCU antibody (Cat# HPA016480, 1:200, Sigma, USA). GAPDH (Cat# ab8245, 1:5000, abcam, USA) was used for Western blot loading control. Detection was performed with the enhanced chemiluminescence reagent (KeyGEN).


A
P = 1.06E-06
N = 61
N = 76
Normal breast
Invasive Bca
B
P = 3.80E-12
N = 61
N = 392
Normal breast
Invasive Ductal Bca
C
P = 7.36E-05
N = 61
N = 36
Normal breast
Invasive Lobular Bca
D
P = 0.001
N = 61
N = 4
Normal breast
Mucinous Bca
E
P = 0.001
N = 61
N = 7
Normal breast
Mixed lobular & ductal Bca
F
P = 7.36E-05
N = 61
N = 3
Normal breast
Intraductal cribriform Bca
G
P= 7.02E-05
N = 3
N = 393
Ductal Bca in situ
Invasive ductal bca
H
P = 0.035
N = 39
N = 32
Log2 Copy Number Units
N0, Invasive lobular bca
N1+, Invasive lobular bca
Log2 Median Centered Ratio

2.9. Statistical analysis

Statistical analysis was performed using the SPSS 13.0 software. Results were expressed as mean ± SEM of the mean. Data were analyzed by Student's t test. The difference was considered to be statistically significant at $P < 0.05$.

3. Results

3.1. Overexpression of MCU is correlated to invasiveness of breast cancer

We examined the Oncomine™ for analysis and visualization of MCU in breast cancer types for its mRNA expression levels in human breast cancer tissues with the methods described [16,17]. The datasets from the Oncomine cancer microarray database were selected to determine the alterations of MCU in mRNA expression levels. MCU mRNA levels were significantly higher in multiple subtypes of breast cancer including invasive, mucinous, and cribriform of breast cancers. Representative analyses are shown for each indicated breast cancer subtypes in mRNA levels (Fig. 1A–F). Invasive ductal breast carcinoma expressed significantly higher MCU mRNA than ductal carcinoma in situ (Fig. 1G). In addition, MCU expression is correlated with lymph node status in breast cancer patients (Fig. 1H).

3.2. MCU inhibition RuR reduces breast cancer cell migration

To determine the role of MCU protein in breast cancer cell migration, we observed the effect of RuR on cell viability by MTT assay. Up to 48 h, MDA-MB-231 cell viability remained unaffected after treatment with different concentration of RuR from 10 to 100 μM (data not shown). Since there was no significant effect observed on cell viability of MDA-MB-231 cells treated by RuR (less than 100 μM) within 48 h, we therefore determined whether MCU is involved in the breast cancer cell migration by wound-healing assays within 48 h observation (Fig. 2A). In the presence of serum, cells would migrate and fill the gap made by a pipette tip after 48 h. The inhibitory effect of RuR on cell migration was observed in different concentration of RuR from 25 to 100 μM (Fig. 2B). Furthermore, using transwell assay, we confirmed that treatment of MDA-MB-231 cells with RuR from 25 to 100 μM decreased the percentage of cells those migrated through the inserts (Fig. 2C). Next , we selectively knocked down MCU protein expression. Transfection of MDA-MB-231 cells with two siRNA-MCUs, but not siRNA-scrambled, substantially blocked the expression of MCU protein (Fig. 2D). We also verified that siRNA-MCU transfections have no obviously effect on cell viability within 24 h (data not shown). Using transwell assay for 18 h observation, we showed that MCU knockdown by either siRNA-MCU inhibited the serum-induced cell migration significantly (Fig. 3E). These data indicate that MCU inhibition by both pharmacological inhibitors and RNAi impairs human breast cancer cell migration.

3.3. MCU inhibition attenuate SOCE

To confirm that both RuR and MCU knockdown block Ca^{2+} influx into mitochondria, rhod-2 AM-based Ca^{2+} measurement was performed. As shown in Fig. 3A, RuR treatment (from 25 to 100 μM) decreased serum-induced mitochondrial Ca^{2+} uptake of MDA-MB-231 cells dose-dependently. In addition, mitochondrial Ca^{2+} measurement showed that both of siRNA-MCUs significantly decreased the mitochondrial Ca^{2+} uptake in MDA-MB-231 cells (Fig. 3B). Previous reports showed that mitochondria regulate SOCE [11]. Therefore, we speculated that MCU modulate breast cancer cell migration by regulating SOCE. We then determined the effects of MCU inhibition on SOCE in MDA-MB-231 cells. MCU inhibition by RuR at 25–100 μM concentration reduced both maximal Ca^{2+} release elevation (the first peak phase) and maximal Ca^{2+} entry elevation (the second peak phase) [21] induced by 10% serum (Fig. 3C). Considering the complicated components of serum-induced Ca^{2+} influx (not only CRAC channel of SOCE but also other Ca^{2+} channels may activated), we determined thapsigargin (TG) to examine the effects of MCU inhibition on SOCE. TG is frequently used to induce SOCE by blocking the sarco-endoplasmic reticulum Ca^{2+}-ATPases (SERCA). We observed that both maximal Ca^{2+} release elevation and maximal Ca^{2+} entry elevation induced by TG (10 μM) were reduced after treating cells were treated with 25–100 μM RuR (Fig. 3D). Furthermore, similar result was observed in TG-stimulated MDA-MB-231 cells which were transfected with both of siRNA-MCUs (Fig. 3E). These results established a role for MCU on SOCE and indicate that SOCE may play a key role in the signaling pathway of MCU-involved breast cancer cell migration.

3.4. SOCE inhibitors block breast cancer cell migration

We have recently shown that SOCE is involved in cell migration [22]. To confirm the role of SOCE in mitochondrial-regulated cell migration, we next determined the effect of SOCE inhibition on MDA-MB-231 cell migration. Cells were pretreated with three SOCE inhibitors, SKF96365, 2-APB [23] and gadolinium (Gd^{3+}) [24]. Fluo 4-based Ca^{2+} measurement was performed to measure Ca^{2+} influx. As shown in Fig. 4A and B, all three SOCE inhibitors decreased serum- or TG-induced maximal calcium entry elevations in MDA-MB-231 cells. Then, using wound-healing assays, we showed that the migration of MDA-MB-231 cells could be blocked by pretreatment of three SOCE inhibitors (Fig. 4C and 4D). Moreover, similar results were also obtained by transwell assays (Fig. 4E). These results indicate that SOCE plays a critical role in cell migration and is involved in MCU-regulated breast cancer cell migration.

4. Discussion

This work addresses the role of MCU in cell migration and breast cancer metastasis. Our data demonstrate that MCU is correlated to metastasis and invasive human breast cancer, and MCU inhibition or silence blocks breast cancer cell migration which essentially depends on SOCE abrogation. The contribution of mitochondrial Ca^{2+} signaling to breast cancer cell migration suggests MCU could be as potential cancer therapeutic targets.

MCU is a major Ca^{2+} channel in mitochondrial inner membrane and its molecular basis was remained elusive until 2011 [10]. Mitochondrial Ca^{2+} increases upregulate aerobic metabolism [25] and sensitize mitochondria to apoptotic challenges [26]. Evidence has showed that mitochondrial depolarization inhibits SOCE through CRAC channels and a major factor contributing to this

Fig. 1. MCU expression is associated with metastasis and invasive ductal breast cancers. Selected TCGA datasets from the Oncomine cancer microarray database (http://www.oncomine.org) were mined to determine the alterations of MCU in mRNA expression levels. Representative datasets from breast cancer subtypes were compared with MCU expression. A. invasive breast carcinoma vs. normal control; B. invasive ductal breast carcinoma vs. normal control; C. invasive lobular breast carcinoma vs. normal control; D. mucinous breast carcinoma vs. normal control; E. mixed lobular and ductal breast carcinoma vs. normal control; F. intraductal cribriform breast carcinoma vs. normal control. G. ductal carcinoma in situ vs. invasive ductal breast carcinoma. H. N0 vs. N1+ of invasive lobular breast carcinoma. T-tests were performed for statistical significance for the entire series of analysis. Log2 median centered values are indicated.

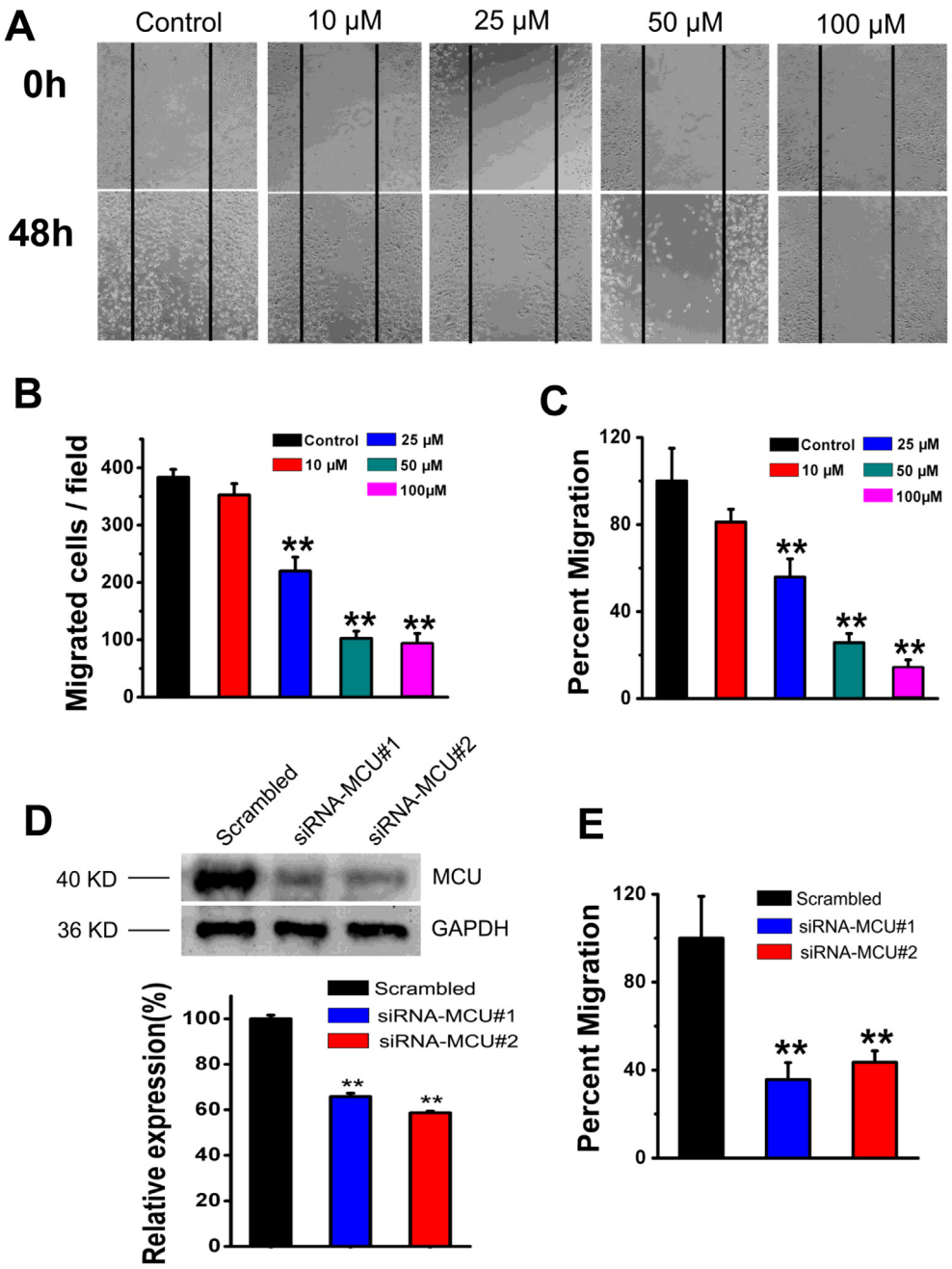


Fig. 2. Effects of MCU inhibition on breast cancer cell migration. A. Representative wound healing images of MDA-MB-231 cells. Cells were treated with RuR at 10–100 μM concentrations for 48 h. B. Quantification of wound healing field (migrated cells). C. Transwell assay to measure cell migration to serum in MDA-MB-231 cells. Cells were treated by RuR at 10–100 μM concentrations and seeded to transwell inserts for 18 h incubation. D. The expression of MCU protein after RNA inference. MDA-MB-231 cells were transfected for 48 h with siRNA-scrambled, siRNA-MCU#1 or siRNA-MCU#2. Cells were harvested, total protein were extracted and subjected to Western blotting analysis with antibodies anti-MCU and anti-GAPDH as loading control. E. Transwell assay to measure cell migration to serum in MDA-MB-231 cells. Cells were transfected for 48 h before seeded to transwell inserts and incubated for 18 h at 37 °C. Data shown are representative of 3 independent experiments. Data are mean ± SEM. $^{**}p < 0.01$.

effect involves mitochondrial buffering of cytoplasmic Ca^{2+} [27]. The recent studies identified that MCU Is Dispensable for MDA-MB-231 cell survival [28] and downregulation of MCU potentiated caspase-independent cell death [29]. However, evidence is limited regarding the effects of mitochondria calcium transporter on cell migration. Our new finding was the first report to demonstrate that MCU is responsible for cancer cell migration.

Indeed, intracellular Ca^{2+} signaling is important for cancer cell migration [30]. Ca^{2+} signals have both global and local regulatory roles on cell motility, because they target the contractile proteins as well as many regulatory proteins. The major Ca^{2+} entry pathway in non-excitable cells in general, and cancer cells in particular, is provided by SOCE [31]. We confirmed that the inhibitions of store-operated Ca^{2+} entry (SOCE) by pharmacological agents, SKF96365, 2-APB or Gd^{3+}, were able to suppress MDA-MB-231 human breast cancer cell migration. This result is consistent with previous research which reports that Orai1 and STIM1, two key molecules responsible for SOCE, are required for breast cancer cell migration [5]. Moreover, downregulation of transcription factor Oct4 enhances SOCE in breast cancer cells and hence induces an epithelial-to-mesenchymal transition, which is one of the most critical processes that occur during the progression of cancer metastasis [32].

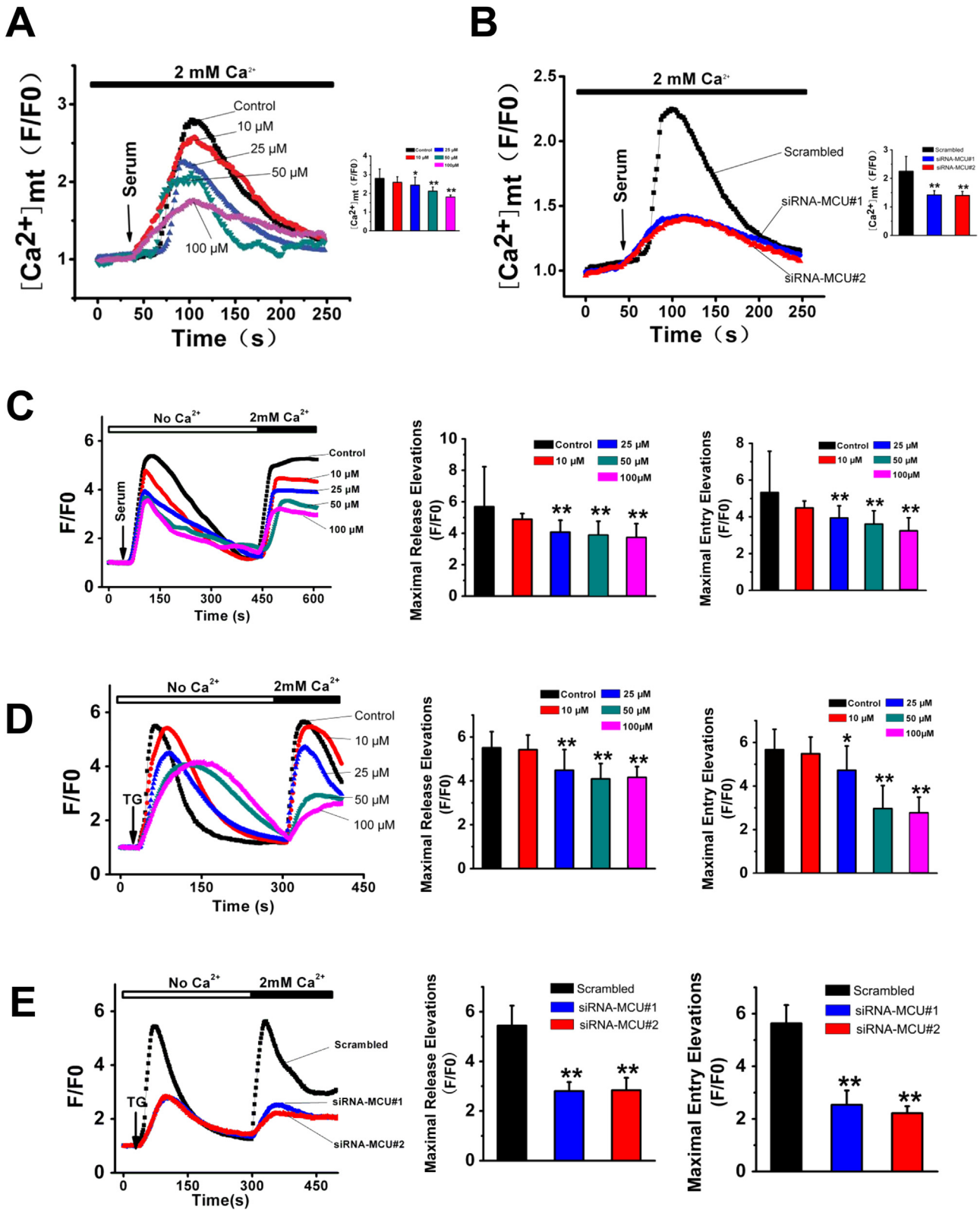


Fig. 3. Effect of MCU inhibition on SOCE in breast cancer cells. A. RuR treatment dramatically reduces mitochondrial Ca^{2+} uptake. MDA-MB-231 cells were treated with RuR at 10–100 μM concentrations for 18 h. B. MCU silencing dramatically reduces mitochondrial Ca^{2+} uptake. The MCU siRNAs were transfected 48 h before the $[Ca^{2+}]_{mt}$ measurements. RuR treatment substantially reduces serum-stimulated (C) or TG-stimulated (D) intracellular Ca^{2+} release and extracellular Ca^{2+} entry. MDA-MB-231 cells were treated with RuR at 10–100 μM concentrations. E. MCU siRNA transfections reduce TG-stimulated intracellular Ca^{2+} release and extracellular Ca^{2+} entry. The left panel depicts relative $[Ca^{2+}]_{mt}$ (F/F0) (A, B) or $[Ca^{2+}]_i$ (F/F0) (C, D, E) represented as response over baseline. The right panels represent pooled data for the peak F/F0 ($N = 15$) consisting of the maximal response over baseline value during the Ca^{2+} influx transient. Data shown are representative of 3 independent experiments. Data are mean ± SEM. *$p < 0.05$, **$p < 0.01$.

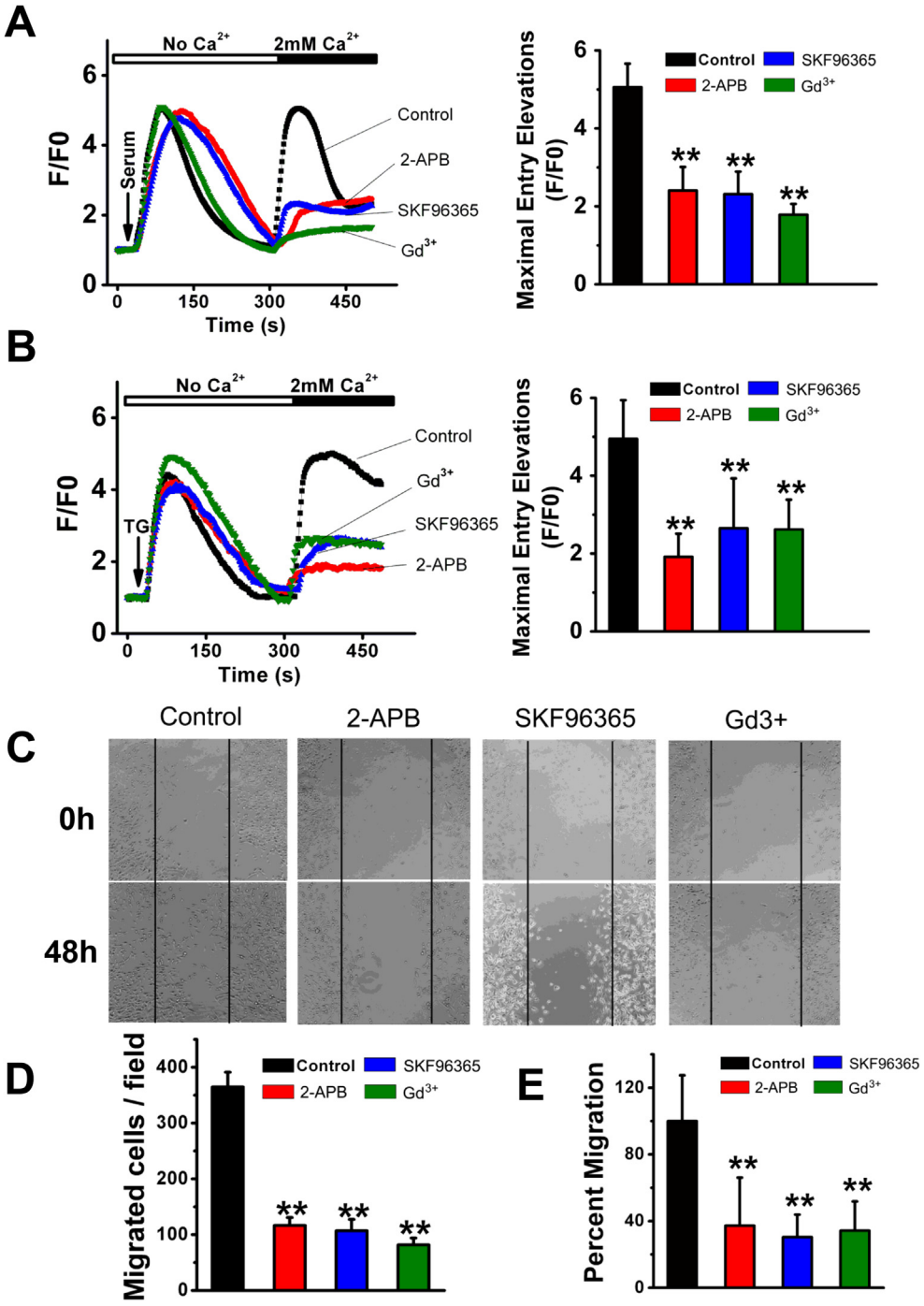


Fig. 4. Effects of SOCE inhibitions on breast cancer cell migration. MDA-MB-231 cells were pretreated with SKF96365 (10 μM), 2-APB (100 μM) or $GdCl_3$ (2 μM) for 20 min. SOCE inhibitions reduce serum-stimulated (A) or TG-stimulated (B) extracellular Ca^{2+} entry. The left panel depicts relative $[Ca^{2+}]_i$ (F/F0) represented as response over baseline. The arrow corresponds to the addition of serum and TG. The right panel represents pooled data for the peak F/F0 (N = 15) consisting of the maximal response over baseline value during extracellular Ca^{2+} influx transient. C. Representative wound healing images of MDA-MB-231 cells. D. Quantification of wound healing field (migrated cells). E. Transwell assay to measure cell migration to serum in MDA-MB-231 cells. Cells were seeded to transwell inserts for 18 h incubation. Data shown are representative of 3 independent experiments. Data are mean ± SEM. $^{**}p < 0.01$.

These data suggest that SOCE participates the process of breast cancer cell migration and is regulated by different molecules. Better understanding of its upstream regulators will contribute to prevent and treat breast cancer metastasis.

Mitochondria are key regulators of SOCE, a ubiquitous Ca^{2+} influx pathway, mainly by dynamic interplay with endoplasmic reticulum [33,34], biphasic regulation of cytosolic Ca^{2+} concentration [35], accelerating store refilling and thus promoting deactivation of the CRAC channels [36]. However, the molecular mechanism remains unclear. Taking into account that cytosolic Ca^{2+} concentration is regulated by mitochondria, we explored the effects of MCU inhibition and silence on SOCE. Our evidence demonstrated at molecular level that MCU is responsible for mitochondrial regulation of SOCE.

Based on these studies, we conclude that MCU is required for SOCE-mediated breast cancer cell migration. MCU expression levels in cancer cells may affect Ca^{2+} homeostasis and hence result in

distinct cancer metastasis and prognosis. Considering different expression levels of MCU proteins in cancer cells may indicate the prognosis of patients, further works should be done to determine whether MCU can be a biomarker for cancer. Moreover, agents that block MCU or antibodies that specifically block the channel activity of MCU are attractive for therapeutic intervention of cancer metastasis.

Conflict of interest

All authors including Shihao Tang, Xubu Wang, Qiang Shen, Xinyi Yang, Changhui Yu, Chunqing Cai, Guoshuai Cai, Xiaojing Meng, and Fei Zou declare that there are no conflicts of interest.

Acknowledgment

This work was supported by grants from National Basic Research Program of China, No. 2012CB525004 (XM); No. 2012CB518200 (FZ), National Natural Science Foundation of China No. 81272180 (FZ).

References

[1] A. Jemal, F. Bray, M.M. Center, J. Ferlay, E. Ward, D. Forman, Global cancer statistics, CA Cancer J. Clin. 61 (2011) 69–90.
[2] G.R. Monteith, D. McAndrew, H.M. Faddy, S.J. Roberts-Thomson, Calcium and cancer: targeting Ca2+ transport, Nat. Rev. Cancer 7 (2007) 519–530.
[3] H.L. Roderick, S.J. Cook, Ca2+ signalling checkpoints in cancer: remodelling Ca2+ for cancer cell proliferation and survival, Nat. Rev. Cancer 8 (2008) 361–375.
[4] T.A. Stewart, K.T. Yapa, G.R. Monteith, Altered calcium signaling in cancer cells, Biochim. Biophys. Acta (2014 Aug 20) pii: S0005-2736(14)00298-3. http://dx.doi.org/10.1016/j.bbamem.2014.08.016. [Epub ahead of print].
[5] S. Yang, J.J. Zhang, X.Y. Huang, Orai1 and STIM1 are critical for breast tumor cell migration and metastasis, Cancer Cell. 15 (2009) 124–134.
[6] S. Yang, X.Y. Huang, Ca2+ influx through L-type Ca2+ channels controls the trailing tail contraction in growth factor-induced fibroblast cell migration, J. Biol. Chem. 280 (2005) 27130–27137.
[7] G. Szabadkai, M.R. Duchen, Mitochondria: the hub of cellular Ca2+ signaling, Physiol. (Bethesda) 23 (2008) 84–94.
[8] S. Pan, S.Y. Ryu, S.S. Sheu, Distinctive characteristics and functions of multiple mitochondrial Ca2+ influx mechanisms, Sci. China Life Sci. 54 (2011) 763–769.
[9] J.M. Baughman, F. Perocchi, H.S. Girgis, M. Plovanich, C.A. Belcher-Timme, Y. Sancak, X.R. Bao, L. Strittmatter, O. Goldberger, R.L. Bogorad, V. Koteliansky, V.K. Mootha, Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter, Nature 476 (2011) 341–345.
[10] D. De Stefani, A. Raffaello, E. Teardo, I. Szabo, R. Rizzuto, A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter, Nature 476 (2011) 336–340.
[11] A.B. Parekh, Mitochondrial regulation of store-operated CRAC channels, Cell. Calcium 44 (2008) 6–13.
[12] S. Naghdi, M. Waldeck-Weiermair, I. Fertschai, M. Poteser, W.F. Graier, R. Malli, Mitochondrial Ca2+ uptake and not mitochondrial motility is required for STIM1-Orai1-dependent store-operated Ca2+ entry, J. Cell. Sci. 123 (2010) 2553–2564.
[13] M.D. Glitsch, D. Bakowski, A.B. Parekh, Store-operated Ca2+ entry depends on mitochondrial Ca2+ uptake, EMBO J. 21 (2002) 6744–6754.
[14] R.S. Lewis, The molecular choreography of a store-operated calcium channel, Nature 446 (2007) 284–287.
[15] A. Vichalkovski, I. Kotevic, N. Gebhardt, R. Kaderli, H. Porzig, Tyrosine kinase modulation of protein kinase C activity regulates G protein-linked Ca2+ signaling in leukemic hematopoietic cells, Cell. Calcium 39 (2006) 517–528.
[16] D.R. Rhodes, S. Kalyana-Sundaram, V. Mahavisno, R. Varambally, J. Yu, B.B. Briggs, T.R. Barrette, M.J. Anstet, C. Kincead-Beal, P. Kulkarni, S. Varambally, D. Ghosh, A.M. Chinnaiyan, Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles, Neoplasia 9 (2007) 166–180.
[17] D.R. Rhodes, S. Kalyana-Sundaram, S.A. Tomlins, V. Mahavisno, N. Kasper, R. Varambally, T.R. Barrette, D. Ghosh, S. Varambally, A.M. Chinnaiyan, Molecular concepts analysis links tumors, pathways, mechanisms, and drugs, Neoplasia 9 (2007) 443–454.
[18] C. Maack, S. Cortassa, M.A. Aon, A.N. Ganesan, T. Liu, B. O'Rourke, Elevated cytosolic Na+ decreases mitochondrial Ca2+ uptake during excitation-contraction coupling and impairs energetic adaptation in cardiac myocytes, Circ. Res. 99 (2006) 172–182.
[19] D. Shan, L. Chen, J.T. Njardarson, C. Gaul, X. Ma, S.J. Danishefsky, X.Y. Huang, Synthetic analogues of migrastatin that inhibit mammary tumor metastasis in mice, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 3772–3776.
[20] X. Wang, C. Li, Y. Chen, Y. Hao, W. Zhou, C. Chen, Z. Yu, Hypoxia enhances CXCR4 expression favoring microglia migration via HIF-1alpha activation, Biochem. Biophys. Res. Commun. 371 (2008) 283–288.
[21] M.J. Berridge, M.D. Bootman, H.L. Roderick, Calcium signalling: dynamics, homeostasis and remodelling, Nat. Rev. Mol. Cell. Biol. 4 (2003) 517–529.
[22] W. Zou, X. Meng, C. Cai, M. Zou, S. Tang, X. Chu, X. Wang, F. Zou, Store-operated Ca2+ entry (SOCE) plays a role in the polarization of neutrophil-like HL-60 cells by regulating the activation of Akt, Src, and Rho family GTPases, Cell. Physiol. Biochem. 30 (2012) 221–237.
[23] J.M. Tang, J. Yuan, Q. Li, J.N. Wang, X. Kong, F. Zheng, L. Zhang, L. Chen, L.Y. Guo, Y.H. Huang, J.Y. Yang, S.Y. Chen, Acetylcholine induces mesenchymal stem cell migration via Ca2+/PKC/ERK1/2 signal pathway, J. Cell. Biochem. 113 (2012) 2704–2713.
[24] M.D. Salmon, J. Ahluwalia, Pharmacology of receptor operated calcium entry in human neutrophils, Int. Immunopharmacol. 11 (2011) 145–148.
[25] J.G. McCormack, A.P. Halestrap, R.M. Denton, Role of calcium ions in regulation of mammalian intramitochondrial metabolism, Physiol. Rev. 70 (1990) 391–425.
[26] G. Kroemer, J.C. Reed, Mitochondrial control of cell death, Nat. Med. 6 (2000) 513–519.
[27] K. Singaravelu, C. Nelson, D. Bakowski, O.M. de Brito, S.W. Ng, J. Di Capite, T. Powell, L. Scorrano, A.B. Parekh, Mitofusin 2 regulates STIM1 migration from the Ca2+ store to the plasma membrane in cells with depolarized mitochondria, J. Biol. Chem. 286 (2011) 12189–12201.
[28] D.D. Hall, Y. Wu, F.E. Domann, D.R. Spitz, M.E. Anderson, Mitochondrial calcium uniporter activity is dispensable for MDA-MB-231 breast carcinoma cell survival, PLoS One 9 (2014) e96866.
[29] M.C. Curry, A.A. Peters, P.A. Kenny, S.J. Roberts-Thomson, G.R. Monteith, Mitochondrial calcium uniporter silencing potentiates caspase-independent cell death in MDA-MB-231 breast cancer cells, Biochem. Biophys. Res. Commun. 434 (2013) 695–700.
[30] N. Prevarskaya, R. Skryma, Y. Shuba, Calcium in tumour metastasis: new roles for known actors, Nat. Rev. Cancer 11 (2011) 609–618.
[31] A. Berna-Erro, P.C. Redondo, J.A. Rosado, Store-operated Ca(2+) entry, Adv. Exp. Med. Biol. 740 (2012) 349–382.
[32] J. Hu, K. Qin, Y. Zhang, J. Gong, N. Li, D. Lv, R. Xiang, X. Tan, Downregulation of transcription factor Oct4 induces an epithelial-to-mesenchymal transition via enhancement of Ca2+ influx in breast cancer cells, Biochem. Biophys. Res. Commun. 411 (2011) 786–791.
[33] G. Szabadkai, K. Bianchi, P. Varnai, D. De Stefani, M.R. Wieckowski, D. Cavagna, A.I. Nagy, T. Balla, R. Rizzuto, Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca2+ channels, J. Cell. Biol. 175 (2006) 901–911.
[34] G. Csordas, C. Renken, P. Varnai, L. Walter, D. Weaver, K.F. Buttle, T. Balla, C.A. Mannella, G. Hajnoczky, Structural and functional features and significance of the physical linkage between ER and mitochondria, J. Cell. Biol. 174 (2006) 915–921.
[35] B. Moreau, C. Nelson, A.B. Parekh, Biphasic regulation of mitochondrial Ca2+ uptake by cytosolic Ca2+ concentration, Curr. Biol. 16 (2006) 1672–1677.
[36] J.A. Gilabert, D. Bakowski, A.B. Parekh, Energized mitochondria increase the dynamic range over which inositol 1,4,5-trisphosphate activates store-operated calcium influx, EMBO J. 20 (2001) 2672–2679.