Original Paper

Lithium Sensitivity of Store Operated Ca^{2+} Entry and Survival of Fibroblasts Isolated from Chorea-Acanthocytosis Patients

Lisann Pelzl^a Bhaeldin Elsir^a Itishri Sahu^a Rosi Bissinger^a Yogesh Singh^a
Basma Sukkar^a Sabina Honisch^a Ludger Schoels^{b,c} Mohamed Jemaà^d
Elisabeth Lang^e Alexander Storch^{f,g} Andreas Hermann^{h,i} Christos Stournaras^{a,j}
Florian Lang^{k,l}

^aDepartment of Medicine III, ^bDepartment of Neurology and Hertie Institute for Clinical Brain Research, ^cGerman Center for Neurodegenerative Diseases (DZNE), Research site Tuebingen, Tuebingen Germany; ^dDepartment of Laboratory Medicine, Translational Cancer Research, Lund University, Lund Sweden; ^eDepartment of Psychiatry, University of Basel, Switzerland; ^fDepartment of Neurology, University of Rostock, Rostock, and ^gGerman Center for Neurodegenerative Diseases (DZNE) Rostock/Greifswald, Rostock, ^hDepartment of Neurology and Center for Regenerative Therapies Dresden (CRTD), Technische Universität Dresden, ⁱGerman Center for Neurodegenerative Diseases (DZNE), Research Site Dresden, Germany; ^jDepartment of Biochemistry, University of Crete Medical School, Heraklion, Greece; ^kDepartment of Molecular Medicine II, Heinrich Heine University Duesseldorf, Duesseldorf, ^lDepartment of Physiology I, University of Tuebingen, Tuebingen, Germany

Key Words

Calcium • SOCE • Lithium • Apoptosis • Neurodegeneration • Orai1 • Cell membrane scrambling

Abstract

Background: The widely expressed protein chorein fosters activation of the phosphoinositide 3 kinase (PI3K) pathway thus supporting cell survival. Loss of function mutations of the chorein encoding gene VPS13A (vacuolar protein sorting-associated protein 13A) causes chorea-acanthocytosis (ChAc), a neurodegenerative disorder paralleled by deformations of erythrocytes. In mice, genetic knockout of chorein leads to enhanced neuronal apoptosis. PI3K dependent signalling upregulates Orai1, a pore forming channel protein accomplishing store operated Ca^{2+} entry (SOCE). Increased Orai1 expression and SOCE have been shown to confer survival of tumor cells. SOCE could be up-regulated by lithium. The present study explored, whether SOCE and/or apoptosis are altered in ChAc fibroblasts and could be modified by lithium treatment. **Methods:** Fibroblasts were isolated from ChAc patients and age-matched healthy volunteers. Cytosolic Ca^{2+} activity ($[\text{Ca}^{2+}]_i$) was estimated from Fura-2-fluorescence, SOCE from increase of $[\text{Ca}^{2+}]_i$ following Ca^{2+} re-addition after Ca^{2+} -store depletion with sarcoendoplasmatic Ca^{2+} -ATPase (SERCA) inhibitor thapsigargin (1 μM), and apoptosis from annexin-V/propidium iodide staining quantified in flow cytometry. **Results:** SOCE was significantly smaller in ChAc fibroblasts than in control fibroblasts. Lithium (2 mM, 24 hours)

Prof. Dr. Florian Lang

Department of Physiology I, University of Tuebingen, Gmelinstr. 5, D-72076 Tuebingen (Germany)
Tel. +49 7071 29 72194, Fax +49 7071 29 5618, E-Mail florian.lang@uni-tuebingen.de

significantly increased and Orai1 blocker 2-Aminoethoxydiphenyl Borate (2-APB, 50 μ M, 24 hours) significantly decreased SOCE. Annexin-V-binding and propidium iodide staining were significantly higher in ChAc fibroblasts than in control fibroblasts. In ChAc fibroblasts annexin-V-binding and propidium iodide staining were significantly decreased by lithium treatment, significantly increased by 2-APB and virtually lithium insensitive in the presence of 2-APB. **Conclusions:** In ChAc fibroblasts, downregulation of SOCE contributes to enhanced susceptibility to apoptosis. Both, decreased SOCE and enhanced apoptosis of ChAc fibroblasts can be reversed by lithium treatment.

© 2017 The Author(s)
Published by S. Karger AG, Basel

Introduction

Chorein supports the activation of phosphoinositide-3-kinase (PI3K)-p85-subunit with subsequent activation of ras-related C3 botulinum toxin substrate 1 (Rac1) and p21 protein-activated kinase 1 (PAK), thus fostering actin polymerization and cell survival [1-4]. Loss-of-function mutations of the chorein encoding gene VPS13A (vacuolar protein sorting-associated protein 13A) cause chorea-acanthocytosis (CA) [5-9], characterized by progressive hyperkinetic movement disorder, cognitive dysfunction, behavioural abnormalities, epilepsy, increased plasma creatine kinase concentration, and erythrocyte acanthocytosis [5, 10]. Gene targeted mice lacking functional chorein display erythrocyte shape changes [11], neuronal apoptosis [12] and altered behaviour [12].

Chorein is widely expressed [13-15] and contributes to regulation of platelet activation [15], endothelial cell stiffness [14] and tumor cell survival [4]. Chorein is particularly important for function and survival of neurons and skeletal muscle cells [5, 10]. In platelets, chorein deficiency decreases the number of intracellular granules and compromises platelet degranulation [15]. Chorein deficiency further compromises dopamine release in pheochromocytoma (PC12) cells [16, 17]. In fibroblasts isolated from ChAc-patients cytoskeletal architecture is severely distorted [17].

In rhabdomyosarcoma cells, chorein has been shown to up-regulate store operated Ca^{2+} entry [18] which is accomplished by the pore-forming Ca^{2+} channel subunits Orai1, Orai2 and/or Orai3 [19-23] as well as their regulators STIM1 and/or STIM2 [24-28]. Increases of cytosolic Ca^{2+} activity ($[\text{Ca}^{2+}]_i$) are powerful regulators of cell survival and cell death [29-33]. In bone cells, Orai1 and thus SOCE is up-regulated by lithium [34], which favorably influences the course of neurodegenerative disease [35-42].

The present study explored whether ChAc fibroblasts differ from fibroblasts of healthy individuals in SOCE and apoptosis and whether SOCE and apoptosis of ChAc fibroblasts are sensitive to lithium treatment.

Materials and Methods

Cells

The study has been approved by the Institutional Review Board of the Technische Universität Dresden (EK45022009). Fibroblasts from patients (n=5) and healthy volunteers (n=5) were isolated from human skin biopsy after written consent by the patients and controls. Skin biopsies were minced using sterile techniques and washed twice in phosphate buffered saline (PBS) supplemented with antibiotics (100 U/ml penicillin and 100 mg/ml streptomycin). Explants were placed into 25 cm^2 flasks and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) and antibiotics, as described above. Cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Once the fibroblasts were established, the fungizone was omitted from the medium. Confluent cells were detached by treatment with 0.25% trypsin and 0.05% EDTA for 5 min, and aliquots of separated cells were subcultured with the same medium. Cell cultures between the third and twelfth passages were used in this study [17].

Western Blotting

Orai1 protein abundance was quantified by western blotting. Cells were washed twice with ice cold PBS and suspended in 200 µl ice-cold RIPA lysis buffer (Thermo Fisher Scientific, USA) containing Halt Protease and Halt Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific, USA). Protein concentration was determined using the Bradford assay (BioRad, München, Germany). Fifty - hundred µg of protein were solubilized in sample buffer at 95°C for 5 min. The proteins were separated by a 12% SDS-PAGE in a Glycine-Tris buffer and electro-transferred into Nitrocellulose membranes for 70 min. After blocking with 5% milk in TBST at room temperature for 1-2 h, the membranes were incubated with primary anti-Orai1 antibody (1:1000, Proteintec) and anti-GAPDH antibody (1:2000, Cell Signaling) at 4°C overnight. After washing (TBST), the blots were incubated with secondary anti-rabbit (1:2000, Cell Signaling) antibody for 1 h at room temperature. Protein bands were detected after additional washes (TBST) with an ECL detection reagent (Amersham, Freiburg, Germany) and quantified with Quantity One Software (BioRad, München, Germany). To assign the right protein size we used Protein-Marker VI (PepLab, Erlangen, Germany).

Ca²⁺ measurements

Fura-2 fluorescence was utilized to determine intracellular Ca²⁺ measurements [43]. Cells were loaded with Fura-2/AM (2 µM, Invitrogen, Goettingen, Germany) for 20 min at 37°C. Cells were excited alternatively at 340 nm and 380 nm through an objective (Fluor 40×/1.30 oil) built in an inverted fluorescence microscope (Axiovert 100, Zeiss, Oberkochen, Germany). Emitted fluorescence intensity was recorded at 505 nm. Data were acquired using specialized computer software (Metafluor, Universal Imaging, Downingtown, USA). Cytosolic Ca²⁺ activity was estimated from the 340 nm/380 nm ratio. SOCE was determined by extracellular Ca²⁺ removal and subsequent Ca²⁺ re-addition in the presence of thapsigargin (1 µM, Invitrogen). For quantification of Ca²⁺ entry, the slope (delta ratio/s) and peak (delta ratio) were calculated following re-addition of Ca²⁺.

Experiments were performed with Ringer solution containing (in mM): 125 NaCl, 5 KCl, 1.2 MgSO₄, 2 CaCl₂, 2 Na₂HPO₄, 32 HEPES, 5 glucose, pH 7.4. To reach nominally Ca²⁺-free conditions, experiments were performed using Ca²⁺-free Ringer solution containing (in mM): 125 NaCl, 5 KCl, 1.2 MgSO₄, 2 Na₂HPO₄, 32 HEPES, 0.5 EGTA, 5 glucose, pH 7.4.

Analysis of apoptosis

After incubation, the cells were centrifuged at 1,000 r.p.m. for 5 minutes. Then, cells were co-stained with annexin V-FITC (Immunotools, Friesoythe, Germany) to assess phosphatidylserine exposure and propidium iodide (PI, Sigma-Aldrich) to estimate cell membrane integrity. To this end cells were incubated in 100 µl Ringer solution containing 5 mM Ca²⁺ and 1 µl annexin V-FITC (Immunotools, Friesoythe, Germany), and 0.075 µl PI stock solution (25 mg/ml PI in PBS) for 15 min at 37°C. Then, 100 µl of Ringer solution were added and annexin V-FITC fluorescence as well as PI was determined by flow cytometry using a FACS calibur (BD, Heidelberg, Germany).

Statistics

Data are expressed as arithmetic means ± SEM. Statistical analysis was made by unpaired t-test or Mann-Whitney test, as appropriate. Only results with p<0.05 were considered statistically significant. All experiments were performed in at least 4 independent replications.

Results

The present study explored whether chorein deficiency in fibroblasts impacts on Orai1 expression and Ca²⁺ signalling. To this end, fibroblasts were isolated from healthy individuals (control fibroblasts) and from patients with chorea-acanthocytosis (ChAc fibroblasts). Orai1 expression was quantified by Western blotting. As illustrated in Fig. 1, fibroblasts from both, ChAc patients and healthy volunteers expressed Orai1. The expression was, however, significantly lower in fibroblasts from ChAc patients than in fibroblasts from healthy volunteers.

Fig. 1. Orai1 abundance in fibroblasts isolated from ChAc patients and healthy volunteers. Representative Western blot (upper panel) and arithmetic means ($\pm$ SEM, $n = 5$ patients and 5 healthy volunteers) of Orai1 protein abundance in fibroblasts isolated from healthy volunteers (white bar) and in fibroblasts isolated from ChAc patients (black bar) ** ($p < 0.01$) indicates statistically significant difference from healthy fibroblasts (two-tailed unpaired t -test).

Fig. 2. Intracellular Ca^{2+} release and store-operated Ca^{2+} entry (SOCE) in fibroblasts isolated from ChAc patients and healthy volunteers. A. Representative tracings of Fura-2 fluorescence-ratio in fluorescence spectrometry before and following extracellular Ca^{2+} removal and addition of thapsigargin ($1 \mu\text{M}$), as well as re-addition of extracellular Ca^{2+} in fibro-

blasts isolated from healthy volunteers (white squares) and from chorea-acanthocytosis (ChAc) patients. B,C. Arithmetic means ($\pm$ SEM, $n = 48$ -61 cells from 4-5 individuals) of peak (B) and slope (C) increase of Fura-2-fluorescence-ratio following addition of thapsigargin ($1 \mu\text{M}$) in fibroblasts isolated from healthy volunteers (white bars) and in fibroblasts isolated from ChAc patients (black bars) D,E. Arithmetic means ($\pm$ SEM, $n = 48$ -61 cells from 4-5 individuals) of peak (D) and slope (E) increase of fura-2-fluorescence-ratio following re-addition of extracellular Ca^{2+} in fibroblasts isolated from healthy volunteers (white bars) and in fibroblasts isolated from ChAc patients (black bars). ** ($p < 0.01$) indicates statistically significant difference from healthy individuals (two-tailed unpaired t -test).

Fura2-fluorescence was taken as a measure of cytosolic Ca^{2+} activity ($[\text{Ca}^{2+}]_i$). In order to test whether chorein deficiency influences intracellular Ca^{2+} release and store-operated Ca^{2+} entry (SOCE), intracellular Ca^{2+} stores were depleted by exposure of the cells to Ca^{2+} -free solutions containing the sarco-/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) inhibitor thapsigargin ($1 \mu\text{M}$) followed by re-addition of extracellular Ca^{2+} in the continued presence

Fig. 3. Intracellular Ca^{2+} release and store-operated Ca^{2+} entry (SOCE) in fibroblasts isolated from ChAc patients with or without lithium or 2-APB treatment. A. Representative tracings of Fura-2 fluorescence-ratio in fluorescence spectrometry before and following extracellular Ca^{2+} removal and addition of thapsigargin (1 μM), as well as re-addition of extracellular Ca^{2+} in fibroblasts isolated from chorea-acanthocytosis (ChAc) patients in the continued presence of thapsigargin and absence of drugs (white circles), in the presence of lithium (black circles) and in the presence of 2-APB (grey circles). B,C. Arithmetic means ($\pm$ SEM, $n = 23$ -73 cells from 4-5 individuals) of peak (B) and slope (C) increase of fura-2-fluorescence-ratio following addition of thapsigargin (1 μM) in fibroblasts isolated from chorea-acanthocytosis (ChAc) patients in the absence of drugs (white bars), in the presence of lithium (black bars) and in the presence of 2-APB (grey bars). D,E. Arithmetic means ($\pm$ SEM, $n = 23$ -73 cells from 4-5 individuals) of peak (D) and slope (E) increase of fura-2-fluorescence-ratio following re-addition of extracellular Ca^{2+} in fibroblasts isolated from chorea-acanthocytosis (ChAc) patients in the absence of drugs (white bars), in the presence of lithium (black bars) and in the presence of 2-APB (grey bars). * ($p < 0.05$), ** ($p < 0.01$) indicates statistically significant difference from untreated cells, §§ ($p < 0.01$) indicates statistically significant difference to respective value in absence of 2-APB, (ANOVA).

of thapsigargin. The Fura2-fluorescence ratio prior to extracellular Ca^{2+} removal was slightly, but significantly ($p < 0.05$) higher in control fibroblasts (0.64 ± 0.03 , $n = 6$) than in ChAc fibroblasts (0.54 ± 0.02 , $n = 5$). As illustrated in Fig. 2, the addition of thapsigargin triggered Ca^{2+} release from intracellular stores, leading to a rapid, transient increase in $[\text{Ca}^{2+}]_i$. The increase of intracellular Ca^{2+} activity following thapsigargin treatment was similar in control fibroblasts and ChAc fibroblasts (Fig. 2). The addition of extracellular Ca^{2+} in the continued presence of thapsigargin was followed by a rapid increase of Fura2-fluorescence in both, control fibroblasts and ChAc fibroblasts, reflecting store operated Ca^{2+} entry (SOCE). The peak of SOCE was significantly lower in ChAc fibroblasts than in control fibroblasts (Fig. 1).

A further series of experiments explored whether SOCE could be restored by lithium treatment (2 mM, 24 hours). Again, intracellular Ca^{2+} stores were depleted by exposure of the cells to Ca^{2+} -free solutions containing the sarco-/endoplasmic reticulum Ca^{2+} /ATPase (SERCA) inhibitor thapsigargin (1 μM) followed by re-addition of extracellular Ca^{2+} in the continued presence of thapsigargin. As shown in Fig. 3, the increase of intracellular Ca^{2+} activity following thapsigargin treatment was significantly higher in lithium treated than

Fig. 4. Phosphatidylserine translocation and propidium iodide uptake in fibroblasts isolated from ChAc patients and healthy volunteers. A,B. Representative dot blots of propidium iodide staining versus annexin-V-binding of fibroblasts isolated from healthy volunteers (A) and from chorea-acanthocytosis (ChAc) patients (B). C. Arithmetic means ($\pm$ SEM, $n = 4$ -5 individuals) of the percentage of annexin-V-binding fibroblasts from healthy volunteers (white bars) and from ChAc patients (black bars), both expressed in percent of the value in healthy volunteers. D. Arithmetic means ($\pm$ SEM, $n = 4$ -5 individuals) of the percentage of propidium iodide staining fibroblasts from healthy volunteers (white bars) and from ChAc patients (black bars), both expressed in percent of the value in healthy volunteers. * ($p < 0.01$) ** ($p < 0.01$) indicates statistically significant difference from healthy individuals (two-tailed unpaired t -test).

in untreated ChAc fibroblasts (Fig. 3). The addition of extracellular Ca^{2+} in the continued presence of thapsigargin was followed by a rapid increase of Fura2-fluorescence, which was again significantly higher in lithium treated than in untreated ChAc fibroblasts (Fig. 3). Both peak and slope of SOCE in ChAc fibroblasts were significantly decreased by Orai1 blocker 2-APB (50 μM) (Fig. 3).

In order to test whether the differences in Ca^{2+} signalling are paralleled by differences in apoptosis between ChAc fibroblasts and control fibroblasts, phosphatidylserine exposing cells were identified utilizing annexin-V-binding, and cells with permeable cell membrane utilizing propidium iodide. Both were quantified by flow cytometry. As illustrated in Fig. 4, the percentage annexin-V-binding ChAc fibroblasts was significantly higher than the percentage annexin-V-binding control fibroblasts. Moreover, the percentage of propidium iodide stained fibroblasts was significantly higher in ChAc cells than in control fibroblasts.

Again, additional experiments explored whether phosphatidylserine translocation could be decreased by lithium treatment (2 mM, 24 hours). As shown in Fig. 5, lithium treatment markedly decreased the annexin-V-binding and propidium iodide staining in ChAc fibroblasts. Treatment of the fibroblasts with Orai1 blocker 2-APB (50 μM) was followed by a significant increase of both, annexin-V-binding and propidium iodide staining. In the presence of 2-APB lithium was without significant effect on annexin-V-binding and propidium iodide staining in ChAc fibroblasts (Fig. 5).

Discussion

The present study reveals a novel pathophysiological element in chorea-acanthocytosis, i.e. deranged regulation of store operated Ca^{2+} entry (SOCE). SOCE is significantly down-regulated

Fig. 5. Phosphatidylserine translocation and propidium iodide uptake in fibroblasts from healthy volunteers and from ChAc patients without and with treatment with lithium and/or 2-APB. A-E. Representative dot blots of propidium iodide staining versus annexin-V-binding of fibroblasts isolated from healthy volunteers (A) and from chorea-acanthocytosis (ChAc) patients (B-E) without treatment (B) and with treatment with lithium (2 mM) alone (C) with 50 μ M 2-APB alone (D) and with lithium and 2-APB (E). F. Arithmetic means ($\pm$ SEM, $n = 4$ -5 individuals) of the percentage annexin-V-binding fibroblasts from healthy volunteers (white bars) and from ChAc patients without (black bars) or with (grey bars) 2 mM lithium treatment without (left bars) and with (right bars) presence of 50 μ M 2-APB (2-APB). Values are expressed in percent of the value in untreated ChAc fibroblasts. G. Arithmetic means ($\pm$ SEM, $n = 4$ -5 individuals) of the percentage propidium iodide staining of fibroblasts from healthy volunteers (white bars) and from ChAc patients without (black bars) or with (grey bars) 2 mM lithium treatment without (left bars) and with (right bars) presence of 50 μ M 2-APB (2-APB). Values are expressed in percent of the value in untreated ChAc fibroblasts. *($p < 0.05$), ***($p < 0.001$) indicates statistically significant difference from untreated ChAc cells, §§($p < 0.01$), §§§($p < 0.01$) indicates statistically significant difference to respective value in absence of 2-APB, (ANOVA).

in fibroblasts lacking functional chorein. Inhibition of Orai1 with 2-APB triggers suicidal death of fibroblasts from chorea-acanthocytosis (ChAc) patients. SOCE thus apparently confers survival of the fibroblasts. Up-regulation of SOCE favors oscillations of cytosolic Ca^{2+} activity ($[\text{Ca}^{2+}]_i$) [44]. The oscillations of $[\text{Ca}^{2+}]_i$ result from rapid Ca^{2+} entry due to activation of SOCE followed by similarly rapid Ca^{2+} extrusion. The repetitive short pulses of $[\text{Ca}^{2+}]_i$ increase activate Ca^{2+} dependent transcription factors and reorganize the actin filament network without triggering the detrimental consequences of sustained increases of $[\text{Ca}^{2+}]_i$ [45]. The Ca^{2+} oscillations participate in the regulation of diverse cellular functions [46-50] including entry into the S and the M phase of the cell cycle [51, 52] and cell survival [53, 54]. In contrast to Ca^{2+} oscillations, sustained increases of cytosolic Ca^{2+} activity trigger apoptosis [48, 55-63]. Along those lines, Orai1, 2, or 3 [19-22], and their regulators STIM 1 or 2 [25, 26, 28] are part of the machinery regulating survival, proliferation, and migration of tumor cells [64-77].

The present study did not address the signalling linking chorein and fibroblast survival. The signalling may well involve PI3K which confers survival of a wide variety of cells including cancer cells [78-92] and neurons [93-96]. PI3K dependent signalling includes the kinases Akt and SGK1 [97], which are both known to up-regulate Orai1 [98, 99].

The present observations further uncover an anti-apoptotic effect of lithium in fibroblasts from chorea-acanthocytosis patients. The effect of lithium is at least in part due to up-regulation of SOCE. Lithium has previously been shown to counteract neurodegeneration [35-42]. In view of the present observations, it is tempting to speculate that lithium may

modify the course of neurodegeneration in part by up-regulating neuronal Orai1 expression and SOCE. As a matter of fact, similar observations on Orai1 expression and SOCE have been made in neurons generated from fibroblasts of ChAc patients [100].

In conclusion, the pathophysiology of chorea-acanthocytosis includes downregulation of the store operated Ca^{2+} entry leading to compromised cell survival. Lithium up-regulates store operated Ca^{2+} entry and attenuates apoptosis.

Acknowledgements

This study was supported by the Brigitte-Schlieben-Lange-Programm (L.P.), DAAD (I.S.) and the Open Access Publishing Fund of Tuebingen University. Work of R.B. is supported by the Institutional Strategy of the University of Tübingen (Deutsche Forschungsgemeinschaft, ZUK63). The work was supported in part by the Advocacy for Neuroacanthocytosis patients (to A.H. and A.S.) and by the Federal Ministry of Education and Research (BMBF FKZ: 01GM1303) under the frame of E-Rare-2, the ERA-Net for Research on Rare Diseases (EMINA2 consortium, to A.H.). The authors acknowledge the meticulous preparation of the manuscript by Lejla Subasic.

Disclosure Statement

None.

References

- 1 IntAct Dfif: [<http://www.ebi.ac.uk/intact/pages/interactions/interactions.xhtml?conversationContext=1>]. EMBL EBI database 2011.
- 2 Wu C, Ma MH, Brown KR, Geisler M, Li L, Tzeng E, Jia CY, Jurisica I, Li SS: Systematic identification of SH3 domain-mediated human protein-protein interactions by peptide array target screening. *Proteomics* 2007;7:1775-1785.
- 3 Foller M, Hermann A, Gu S, Alesutan I, Qadri SM, Borst O, Schmidt EM, Schiele F, vom Hagen JM, Saft C, Schols L, Lerche H, Stournaras C, Storch A, Lang F: Chorein-sensitive polymerization of cortical actin and suicidal cell death in chorea-acanthocytosis. *FASEB J* 2012;26:1526-1534.
- 4 Honisch S, Yu W, Liu G, Alesutan I, Towhid ST, Tsapara A, Schleicher S, Handgretinger R, Stournaras C, Lang F: Chorein addiction in VPS13A overexpressing rhabdomyosarcoma cells. *Oncotarget* 2015;6:10309-10319.
- 5 Dobson-Stone C, Rampoldi L, Bader B, Velayos BA, Walker RH, Danek A, Monaco AP: Choreia-Acanthocytosis. *Gene Rev* 1993;updated 2010.
- 6 Dobson-Stone C, Danek A, Rampoldi L, Hardie RJ, Chalmers RM, Wood NW, Bohlega S, Dotti MT, Federico A, Shizuka M, Tanaka M, Watanabe M, Ikeda Y, Brin M, Goldfarb LG, Karp BI, Mohiddin S, Fananapazir L, Storch A, Fryer AE, Maddison P, Sibon I, Trevisol-Bittencourt PC, Singer C, Caballero IR, Aasly JO, Schmierer K, Dengler R, Hiersemenzel LP, Zeviani M, Meiner V, Lossos A, Johnson S, Mercado FC, Sorrentino G, Dupre N, Rouleau GA, Volkmann J, Arpa J, Lees A, Geraud G, Chouinard S, Nemeth A, Monaco AP: Mutational spectrum of the CHAC gene in patients with chorea-acanthocytosis. *Eur J Hum Genet* 2002;10:773-781.
- 7 Dobson-Stone C, Velayos-Baeza A, Filippone LA, Westbury S, Storch A, Erdmann T, Wroe SJ, Leenders KL, Lang AE, Dotti MT, Federico A, Mohiddin SA, Fananapazir L, Daniels G, Danek A, Monaco AP: Chorein detection for the diagnosis of chorea-acanthocytosis. *Ann Neurol* 2004;56:299-302.
- 8 Ueno S, Maruki Y, Nakamura M, Tomemori Y, Kamae K, Tanabe H, Yamashita Y, Matsuda S, Kaneko S, Sano A: The gene encoding a newly discovered protein, chorein, is mutated in chorea-acanthocytosis. *Nat Genet* 2001;28:121-122.
- 9 Tomiyasu A, Nakamura M, Ichiba M, Ueno S, Saiki S, Morimoto M, Kobal J, Kageyama Y, Inui T, Wakabayashi K, Yamada T, Kanemori Y, Jung HH, Tanaka H, Orimo S, Afawi Z, Blatt I, Aasly J, Ujike H, Babovic-Vuksanovic D, Josephs KA, Tohge R, Rodrigues GR, Dupre N, Yamada H, Yokochi F, Kotschet K, Takei T, Rudzinska M, Szczudlik A, Penco S, Fujiwara M, Tojo K, Sano A: Novel pathogenic mutations and copy number variations

- in the VPS13A Gene in patients with chorea-acanthocytosis. *Am J Med Genet B Neuropsychiatr Genet* 2011;156:620-631.
- 10 Saiki S, Sakai K, Murata KY, Saiki M, Nakanishi M, Kitagawa Y, Kaito M, Gondo Y, Kumamoto T, Matsui M, Hattori N, Hirose G: Primary skeletal muscle involvement in chorea-acanthocytosis. *Mov Disord* 2007;22:848-852.
- 11 Walterfang M, Looi JC, Styner M, Walker RH, Danek A, Niethammer M, Evans A, Kotschet K, Rodrigues GR, Hughes A, Velakoulis D: Shape alterations in the striatum in chorea-acanthocytosis. *Psychiatry Res* 2011;192:29-36.
- 12 Tomemori Y, Ichiba M, Kusumoto A, Mizuno E, Sato D, Muroya S, Nakamura M, Kawaguchi H, Yoshida H, Ueno S, Nakao K, Nakamura K, Aiba A, Katsuki M, Sano A: A gene-targeted mouse model for chorea-acanthocytosis. *J Neurochem* 2005;92:759-766.
- 13 Kurano Y, Nakamura M, Ichiba M, Matsuda M, Mizuno E, Kato M, Agemura A, Izumo S, Sano A: In vivo distribution and localization of chorein. *Biochem Biophys Res Commun* 2007;353:431-435.
- 14 Alesutan I, Seifert J, Pakladok T, Rheinlaender J, Lebedeva A, Towhid ST, Stournaras C, Voelkl J, Schaffer TE, Lang F: Chorein sensitivity of actin polymerization, cell shape and mechanical stiffness of vascular endothelial cells. *Cell Physiol Biochem* 2013;32:728-742.
- 15 Schmidt EM, Schmid E, Munzer P, Hermann A, Eyrych AK, Russo A, Walker B, Gu S, vom Hagen JM, Faggio C, Schaller M, Foller M, Schols L, Gawaz M, Borst O, Storch A, Stournaras C, Lang F: Chorein sensitivity of cytoskeletal organization and degranulation of platelets. *FASEB J* 2013;27:2799-2806.
- 16 Hayashi T, Kishida M, Nishizawa Y, Iijima M, Koriyama C, Nakamura M, Sano A, Kishida S: Subcellular localization and putative role of VPS13A/chorein in dopaminergic neuronal cells. *Biochem Biophys Res Commun* 2012;419:511-516.
- 17 Honisch S, Fehrenbacher B, Lebedeva A, Alesutan I, Castor T, Alkahtani S, Alarifi S, Schaller M, Stournaras C, Lang F: Chorein Sensitive Dopamine Release from Pheochromocytoma (PC12) Cells. *Neurosignals* 2015;23:1-10.
- 18 Yu W, Honisch S, Schmidt S, Yan J, Schmid E, Alkahtani S, Alkahtane AA, Alarifi S, Stournaras C, Lang F: Chorein Sensitive Orai1 Expression and Store Operated Ca²⁺ Entry in Rhabdomyosarcoma Cells. *Cell Physiol Biochem* 2016;40:1141-1152.
- 19 Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG: Orai1 is an essential pore subunit of the CRAC channel. *Nature* 2006;443:230-233.
- 20 Putney JW, Jr.: New molecular players in capacitative Ca²⁺ entry. *J Cell Sci* 2007;120:1959-1965.
- 21 Vig M, Peinelt C, Beck A, Koomoa DL, Rabah D, Koblan-Huberson M, Kraft S, Turner H, Fleig A, Penner R, Kinet JP: CRACM1 is a plasma membrane protein essential for store-operated Ca²⁺ entry. *Science* 2006;312:1220-1223.
- 22 Yeromin AV, Zhang SL, Jiang W, Yu Y, Safrina O, Cahalan MD: Molecular identification of the CRAC channel by altered ion selectivity in a mutant of Orai. *Nature* 2006;443:226-229.
- 23 Zhang SL, Kozak JA, Jiang W, Yeromin AV, Chen J, Yu Y, Penna A, Shen W, Chi V, Cahalan MD: Store-dependent and -independent modes regulating Ca²⁺ release-activated Ca²⁺ channel activity of human Orai1 and Orai3. *J Biol Chem* 2008;283:17662-17671.
- 24 Fahrner M, Muik M, Derler I, Schindl R, Fritsch R, Frischauf I, Romanin C: Mechanistic view on domains mediating STIM1-Orai coupling. *Immunol Rev* 2009;231:99-112.
- 25 Peinelt C, Vig M, Koomoa DL, Beck A, Nadler MJ, Koblan-Huberson M, Lis A, Fleig A, Penner R, Kinet JP: Amplification of CRAC current by STIM1 and CRACM1 (Orai1). *Nat Cell Biol* 2006;8:771-773.
- 26 Penna A, Demuro A, Yeromin AV, Zhang SL, Safrina O, Parker I, Cahalan MD: The CRAC channel consists of a tetramer formed by Stim-induced dimerization of Orai dimers. *Nature* 2008;456:116-120.
- 27 Smyth JT, Hwang SY, Tomita T, DeHaven WI, Mercer JC, Putney JW: Activation and regulation of store-operated calcium entry. *J Cell Mol Med* 2010;14:2337-2349.
- 28 Zhang SL, Yu Y, Roos J, Kozak JA, Deerinck TJ, Ellisman MH, Stauderman KA, Cahalan MD: STIM1 is a Ca²⁺ sensor that activates CRAC channels and migrates from the Ca²⁺ store to the plasma membrane. *Nature* 2005;437:902-905.
- 29 Becchetti A, Arcangeli A: Integrins and ion channels in cell migration: implications for neuronal development, wound healing and metastatic spread. *Adv Exp Med Biol* 2010;674:107-123.
- 30 Burgoyne RD: Neuronal calcium sensor proteins: generating diversity in neuronal Ca²⁺ signalling. *Nat Rev Neurosci* 2007;8:182-193.

- 31 Orrenius S, Zhivotovsky B, Nicotera P: Regulation of cell death: the calcium-apoptosis link. *Nat Rev Mol Cell Biol* 2003;4:552-565.
- 32 Roderick HL, Cook SJ: Ca²⁺ signalling checkpoints in cancer: remodelling Ca²⁺ for cancer cell proliferation and survival. *Nat Rev Cancer* 2008;8:361-375.
- 33 Salter RD, Watkins SC: Dendritic cell altered states: what role for calcium? *Immunol Rev* 2009;231:278-288.
- 34 Zhang B, Yan J, Schmidt S, Salker MS, Alexander D, Foller M, Lang F: Lithium- Sensitive Store-Operated Ca²⁺ Entry in the Regulation of FGF23 Release. *Neurosignals* 2015;23:34-48.
- 35 Aghdam SY, Barger SW: Glycogen synthase kinase-3 in neurodegeneration and neuroprotection: lessons from lithium. *Curr Alzheimer Res* 2007;4:21-31.
- 36 Alvarez G, Munoz-Montano JR, Satrustegui J, Avila J, Bogonez E, Diaz-Nido J: Regulation of tau phosphorylation and protection against beta-amyloid-induced neurodegeneration by lithium. Possible implications for Alzheimer's disease. *Bipolar Disord* 2002;4:153-165.
- 37 Bauer M, Alda M, Priller J, Young LT, International Group For The Study Of Lithium Treated P: Implications of the neuroprotective effects of lithium for the treatment of bipolar and neurodegenerative disorders. *Pharmacopsychiatry* 2003;36 Suppl 3:S250-254.
- 38 Lazzara CA, Kim YH: Potential application of lithium in Parkinson's and other neurodegenerative diseases. *Front Neurosci* 2015;9:403.
- 39 Leeds PR, Yu F, Wang Z, Chiu CT, Zhang Y, Leng Y, Linares GR, Chuang DM: A new avenue for lithium: intervention in traumatic brain injury. *ACS Chem Neurosci* 2014;5:422-433.
- 40 Luo J: Lithium-mediated protection against ethanol neurotoxicity. *Front Neurosci* 2010;4:41.
- 41 Manji HK, Moore GJ, Chen G: Lithium at 50: have the neuroprotective effects of this unique cation been overlooked? *Biol Psychiatry* 1999;46:929-940.
- 42 Manji HK, Moore GJ, Chen G: Lithium up-regulates the cytoprotective protein Bcl-2 in the CNS in vivo: a role for neurotrophic and neuroprotective effects in manic depressive illness. *J Clin Psychiatry* 2000;61 Suppl 9:82-96.
- 43 Yang W, Nurbaeva MK, Schmid E, Russo A, Almilaji A, Szteyn K, Yan J, Faggio C, Shumilina E, Lang F: Akt2- and ETS1-dependent IP3 receptor 2 expression in dendritic cell migration. *Cell Physiol Biochem* 2014;33:222-236.
- 44 Lang F, Friedrich F, Kahn E, Woll E, Hammerer M, Waldegger S, Maly K, Grunicke H: Bradykinin-induced oscillations of cell membrane potential in cells expressing the Ha-ras oncogene. *J Biol Chem* 1991;266:4938-4942.
- 45 Lang F, Shumilina E, Ritter M, Gulbins E, Vereninov A, Huber SM: Ion channels and cell volume in regulation of cell proliferation and apoptotic cell death. *Contrib Nephrol* 2006;152:142-160.
- 46 Berridge MJ, Bootman MD, Roderick HL: Calcium signalling: dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol* 2003;4:517-529.
- 47 Berridge MJ, Bootman MD, Lipp P: Calcium--a life and death signal. *Nature* 1998;395:645-648.
- 48 Berridge MJ, Lipp P, Bootman MD: The versatility and universality of calcium signalling. *Nat Rev Mol Cell Biol* 2000;1:11-21.
- 49 Lang F, Busch GL, Ritter M, Volkl H, Waldegger S, Gulbins E, Haussinger D: Functional significance of cell volume regulatory mechanisms. *Physiol Rev* 1998;78:247-306.
- 50 Parekh AB, Penner R: Store depletion and calcium influx. *Physiol Rev* 1997;77:901-930.
- 51 Steinhardt RA, Alderton J: Intracellular free calcium rise triggers nuclear envelope breakdown in the sea urchin embryo. *Nature* 1988;332:364-366.
- 52 Taylor JT, Zeng XB, Pottle JE, Lee K, Wang AR, Yi SG, Scruggs JA, Sikka SS, Li M: Calcium signaling and T-type calcium channels in cancer cell cycling. *World J Gastroenterol* 2008;14:4984-4991.
- 53 Heise N, Palme D, Misovic M, Koka S, Rudner J, Lang F, Salih HR, Huber SM, Henke G: Non-selective cation channel-mediated Ca²⁺-entry and activation of Ca²⁺/calmodulin-dependent kinase II contribute to G2/M cell cycle arrest and survival of irradiated leukemia cells. *Cell Physiol Biochem* 2010;26:597-608.
- 54 Parkash J, Asotra K: Calcium wave signaling in cancer cells. *Life Sci* 2010;87:587-595.
- 55 Benavides Damm T, Egli M: Calcium's role in mechanotransduction during muscle development. *Cell Physiol Biochem* 2014;33:249-272.
- 56 Fang KM, Chang WL, Wang SM, Su MJ, Wu ML: Arachidonic acid induces both Na⁺ and Ca²⁺ entry resulting in apoptosis. *J Neurochem* 2008;104:1177-1189.

- 57 Lang F, Hoffmann EK: Role of ion transport in control of apoptotic cell death. *Compr Physiol* 2012;2:2037-2061.
- 58 Liu XH, Kirschenbaum A, Yu K, Yao S, Levine AC: Cyclooxygenase-2 suppresses hypoxia-induced apoptosis via a combination of direct and indirect inhibition of p53 activity in a human prostate cancer cell line. *J Biol Chem* 2005;280:3817-3823.
- 59 Shaik N, Zbidah M, Lang F: Inhibition of Ca(2+) entry and suicidal erythrocyte death by naringin. *Cell Physiol Biochem* 2012;30:678-686.
- 60 Spassova MA, Soboloff J, He LP, Hewavitharana T, Xu W, Venkatachalam K, van Rossum DB, Patterson RL, Gill DL: Calcium entry mediated by SOCs and TRP channels: variations and enigma. *Biochim Biophys Acta* 2004;1742:9-20.
- 61 Svoboda N, Pruetting S, Grissmer S, Kerschbaum HH: cAMP-dependent chloride conductance evokes ammonia-induced blebbing in the microglial cell line, BV-2. *Cell Physiol Biochem* 2009;24:53-64.
- 62 Towhid ST, Schmidt EM, Tolios A, Munzer P, Schmid E, Borst O, Gawaz M, Stegmann E, Lang F: Stimulation of platelet death by vancomycin. *Cell Physiol Biochem* 2013;31:102-112.
- 63 Green DR, Reed JC: Mitochondria and apoptosis. *Science* 1998;281:1309-1312.
- 64 Baryshnikov SG, Pulina MV, Zulian A, Linde CI, Golovina VA: Orai1, a critical component of store-operated Ca2+ entry, is functionally associated with Na+/Ca2+ exchanger and plasma membrane Ca2+ pump in proliferating human arterial myocytes. *Am J Physiol Cell Physiol* 2009;297:C1103-1112.
- 65 Bergmeier W, Weidinger C, Zee I, Feske S: Emerging roles of store-operated Ca(2)(+) entry through STIM and ORAI proteins in immunity, hemostasis and cancer. *Channels (Austin)* 2013;7:379-391.
- 66 Berra-Romani R, Mazzocco-Spezia A, Pulina MV, Golovina VA: Ca2+ handling is altered when arterial myocytes progress from a contractile to a proliferative phenotype in culture. *Am J Physiol Cell Physiol* 2008;295:C779-790.
- 67 Capiod T: The need for calcium channels in cell proliferation. *Recent Pat Anticancer Drug Discov* 2013;8:4-17.
- 68 Courjaret R, Machaca K: STIM and Orai in cellular proliferation and division. *Front Biosci (Elite Ed)* 2012;4:331-341.
- 69 Moccia F, Dragoni S, Lodola F, Bonetti E, Bottino C, Guerra G, Laforenza U, Rosti V, Tanzi F: Store-dependent Ca(2+) entry in endothelial progenitor cells as a perspective tool to enhance cell-based therapy and adverse tumour vascularization. *Curr Med Chem* 2012;19:5802-5818.
- 70 Prevarskaya N, Skryma R, Shuba Y: Calcium in tumour metastasis: new roles for known actors. *Nat Rev Cancer* 2011;11:609-618.
- 71 Chen YF, Chiu WT, Chen YT, Lin PY, Huang HJ, Chou CY, Chang HC, Tang MJ, Shen MR: Calcium store sensor stromal-interaction molecule 1-dependent signaling plays an important role in cervical cancer growth, migration, and angiogenesis. *Proc Natl Acad Sci U S A* 2011;108:15225-15230.
- 72 Faouzi M, Hague F, Potier M, Ahidouch A, Sevestre H, Ouadid-Ahidouch H: Down-regulation of Orai3 arrests cell-cycle progression and induces apoptosis in breast cancer cells but not in normal breast epithelial cells. *J Cell Physiol* 2011;226:542-551.
- 73 Flourakis M, Lehen'kyi V, Beck B, Raphael M, Vandenberghe M, Abeele FV, Roudbaraki M, Lepage G, Mauroy B, Romanin C, Shuba Y, Skryma R, Prevarskaya N: Orai1 contributes to the establishment of an apoptosis-resistant phenotype in prostate cancer cells. *Cell Death Dis* 2010;1:e75.
- 74 Motiani RK, Abdullaev IF, Trebak M: A novel native store-operated calcium channel encoded by Orai3: selective requirement of Orai3 versus Orai1 in estrogen receptor-positive versus estrogen receptor-negative breast cancer cells. *J Biol Chem* 2010;285:19173-19183.
- 75 Prevarskaya N, Ouadid-Ahidouch H, Skryma R, Shuba Y: Remodelling of Ca2+ transport in cancer: how it contributes to cancer hallmarks? *Philos Trans R Soc Lond B Biol Sci* 2014;369:20130097.
- 76 Schmid E, Bhandaru M, Nurbaeva MK, Yang W, Sztejn K, Russo A, Leibrock C, Tyan L, Pearce D, Shumilina E, Lang F: SGK3 regulates Ca(2+) entry and migration of dendritic cells. *Cell Physiol Biochem* 2012;30:1423-1435.
- 77 Qu B, Al-Ansary D, Kummerow C, Hoth M, Schwarz EC: ORAI-mediated calcium influx in T cell proliferation, apoptosis and tolerance. *Cell Calcium* 2011;50:261-269.
- 78 Davis NM, Sokolosky M, Stadelman K, Abrams SL, Libra M, Candido S, Nicoletti F, Polesel J, Maestro R, D'Assoro A, Drobot L, Rakus D, Gizak A, Laidler P, Dulinska-Litewka J, Basecke J, Mijatovic S, Maksimovic-Ivanic D, Montalto G, Cervello M, Fitzgerald TL, Demidenko Z, Martelli AM, Cocco L, Steelman LS, McCubrey

- JA: Deregulation of the EGFR/PI3K/PTEN/Akt/mTORC1 pathway in breast cancer: possibilities for therapeutic intervention. *Oncotarget* 2014;
- 79 Ding D, Wei S, Song Y, Li L, Du G, Zhan H, Cao Y: Osteoblast exhibits anti-cancer property in rat glioma cells through inhibiting PI3K/Akt and MAPK signaling pathways. *Cell Physiol Biochem* 2013;32:1751-1760.
- 80 Francipane MG, Lagasse E: mTOR pathway in colorectal cancer: an update. *Oncotarget* 2014;5:49-66.
- 81 Fruman DA, Rommel C: PI3K and cancer: lessons, challenges and opportunities. *Nat Rev Drug Discov* 2014;13:140-156.
- 82 Hirsch E, Ciraolo E, Franco I, Ghigo A, Martini M: PI3K in cancer-stroma interactions: bad in seed and ugly in soil. *Oncogene* 2014;33:3083-3090.
- 83 Hou J, Lam F, Proud C, Wang S: Targeting Mnk for cancer therapy. *Oncotarget* 2012;3:118-131.
- 84 Martelli AM, Chiarini F, Evangelisti C, Cappellini A, Buontempo F, Bressanin D, Fini M, McCubrey JA: Two hits are better than one: targeting both phosphatidylinositol 3-kinase and mammalian target of rapamycin as a therapeutic strategy for acute leukemia treatment. *Oncotarget* 2012;3:371-394.
- 85 Martini M, De Santis MC, Braccini L, Gulluni F, Hirsch E: PI3K/AKT signaling pathway and cancer: an updated review. *Ann Med* 2014;10.3109/07853890.2014.9128361-12.
- 86 McCubrey JA, Steelman LS, Bertrand FE, Davis NM, Sokolosky M, Abrams SL, Montalto G, D'Assoro AB, Libra M, Nicoletti F, Maestro R, Basecke J, Rakus D, Gizak A, Demidenko ZN, Cocco L, Martelli AM, Cervello M: GSK-3 as potential target for therapeutic intervention in cancer. *Oncotarget* 2014;5:2881-2911.
- 87 McCubrey JA, Steelman LS, Chappell WH, Abrams SL, Franklin RA, Montalto G, Cervello M, Libra M, Candido S, Malaponte G, Mazzarino MC, Fagone P, Nicoletti F, Basecke J, Mijatovic S, Maksimovic-Ivanic D, Milella M, Tafuri A, Chiarini F, Evangelisti C, Cocco L, Martelli AM: Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR cascade inhibitors: how mutations can result in therapy resistance and how to overcome resistance. *Oncotarget* 2012;3:1068-1111.
- 88 Ocana A, Vera-Badillo F, Al-Mubarak M, Templeton AJ, Corrales-Sanchez V, Diez-Gonzalez L, Cuenca-Lopez MD, Seruga B, Pandiella A, Amir E: Activation of the PI3K/mTOR/AKT pathway and survival in solid tumors: systematic review and meta-analysis. *PLoS One* 2014;9:e95219.
- 89 Rodon J, Dienstmann R, Serra V, Tabernero J: Development of PI3K inhibitors: lessons learned from early clinical trials. *Nat Rev Clin Oncol* 2013;10:143-153.
- 90 Xiao M, Tang Y, Wang YL, Yang L, Li X, Kuang J, Song GL: ART1 silencing enhances apoptosis of mouse CT26 cells via the PI3K/Akt/NF-kappaB pathway. *Cell Physiol Biochem* 2013;32:1587-1599.
- 91 Zhang H, Guo M, Chen JH, Wang Z, Du XF, Liu PX, Li WH: Osteopontin knockdown inhibits alpha5beta1 integrin-induced cell migration and invasion and promotes apoptosis of breast cancer cells by inducing autophagy and inactivating the PI3K/Akt/mTOR pathway. *Cell Physiol Biochem* 2014;33:991-1002.
- 92 Zhang L, Zhou F, ten Dijke P: Signaling interplay between transforming growth factor-beta receptor and PI3K/AKT pathways in cancer. *Trends Biochem Sci* 2013;38:612-620.
- 93 Sedding DG: FoxO transcription factors in oxidative stress response and ageing--a new fork on the way to longevity? *Biol Chem* 2008;389:279-283.
- 94 Dudek H, Datta SR, Franke TF, Birnbaum MJ, Yao R, Cooper GM, Segal RA, Kaplan DR, Greenberg ME: Regulation of neuronal survival by the serine-threonine protein kinase Akt. *Science* 1997;275:661-665.
- 95 Arboleda G, Morales LC, Benitez B, Arboleda H: Regulation of ceramide-induced neuronal death: cell metabolism meets neurodegeneration. *Brain Res Rev* 2009;59:333-346.
- 96 Kreis P, Rousseau V, Thevenot E, Combeau G, Barnier JV: The four mammalian splice variants encoded by the p21-activated kinase 3 gene have different biological properties. *J Neurochem* 2008;106:1184-1197.
- 97 Raimondi C, Falasca M: Targeting PDK1 in cancer. *Curr Med Chem* 2011;18:2763-2769.
- 98 Lang F, Eylestein A, Shumilina E: Regulation of Orai1/STIM1 by the kinases SGK1 and AMPK. *Cell Calcium* 2012;52:347-354.
- 99 Schmidt S, Liu G, Liu G, Yang W, Honisch S, Pantelakos S, Stournaras C, Honig A, Lang F: Enhanced Orai1 and STIM1 expression as well as store operated Ca²⁺ entry in therapy resistant ovary carcinoma cells. *Oncotarget* 2014;5:4799-4810.
- 100 Pelzl L, Hauser S, Elsir B, Sukkar B, Sahu I, Singh Y, Höflinger P, Bissinger R, Jemaà M, Stournaras C, Schöls L, Lang F: Lithium Sensitive ORAI1 Expression, Store Operated Ca²⁺ Entry and Suicidal Death of Neurons in Chorea-Acanthocytosis. *Sci Rep* 2017;7:6457.