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Altered calcium signaling in cancer cells

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Abstract

It is the nature of the calcium signal, as determined by the coordinated activity of a suite of calcium channels, pumps, exchangers and binding proteins that ultimately guides a cells fate. Deregulation of the calcium signal is often deleterious and has been linked to each of the 'cancer hallmarks'. Despite this, we do not yet have a full understanding of the remodeling of the calcium signal associated with cancer. Such an understanding could aid in guiding the development of therapies specifically targeting altered calcium signaling in cancer cells during tumorigenic progression. Findings from some of the studies that have assessed the remodeling of the calcium signal associated with tumorigenesis and/or processes important in invasion and metastasis are presented in this review. The potential of new methodologies are also discussed.

Keywords

Calcium signaling; calcium remodeling; cancer; calcium channels; calcium pumps; cytosolic free Ca^{2+}

1. Introduction

Tightly controlled regulation of the calcium signal is essential for appropriate cellular functioning, as evidenced by the role of changes in cytosolic free Ca^{2+} in processes such as cell proliferation, gene transcription and cell death [1-5]. Typically cells at rest maintain an intracellular calcium concentration ($[Ca^{2+}]_i$) of approximately 100 nM, while extracellular calcium concentrations are much higher, generally within the range of 1-2 mM [3-5].

Abbreviations: ATP, adenosine triphosphate; EGF, epidermal growth factor; EMT, epithelial-mesenchymal transition; IP_3R2, inositol 1,4,5-triphosphate receptor, type 2; PMCA, plasma membrane Ca^{2+} ATPase; SERCA, sarco/endoplasmic reticulum Ca^{2+} ATPase; SOCE, store operated Ca^{2+} entry; STIM1, stromal interaction molecule 1; TRP, transient receptor potential

Specialized calcium pumps, channels and calcium binding proteins are used by cells to both maintain cellular homeostasis and carry out specific cellular functions, and have been referred to as the "molecular toolkit" for calcium signaling [1, 2] (Fig. 1). Changes in cytosolic free Ca^{2+} can involve global increases that may be transient or sustained, or highly localized such as calcium sparks and puffs, or they may occur as waves or oscillations [1, 5]. These changes can be "decoded" by the cell, which allows the ubiquitous calcium signal to specifically regulate cellular processes [1, 2]. This complexity in calcium signaling means that the deregulation of the calcium signal can be a feature of certain pathological states, including cancer [5-7]. Much of the research assessing calcium signaling in cancer has focused on determining changes in the expression levels of proteins responsible for regulating cytoplasmic free Ca^{2+} concentrations. Following the identification of aberrantly expressed calcium channels, pumps or exchangers, researchers often then rely on gene silencing approaches and/or chemical inhibitors/activators to evaluate their role in calcium signaling and cancer relevant processes (e.g. proliferation and migration). However, in the context of cancer, compared to some other disease states, there is a paucity of information regarding changes in the *nature* of the calcium signal that occurs in cancer cells compared to non-cancer derived cells. Elucidating such information would improve our understanding of the mechanisms underlying cancer progression, and may further help guide researchers to identify molecular targets not associated with changes in expression. This review will discuss the available evidence for the remodeling of the calcium signal in cancer, and briefly describe studies in other disease states to highlight potential approaches that could further improve our understanding of alterations in calcium signaling in cancer cells.

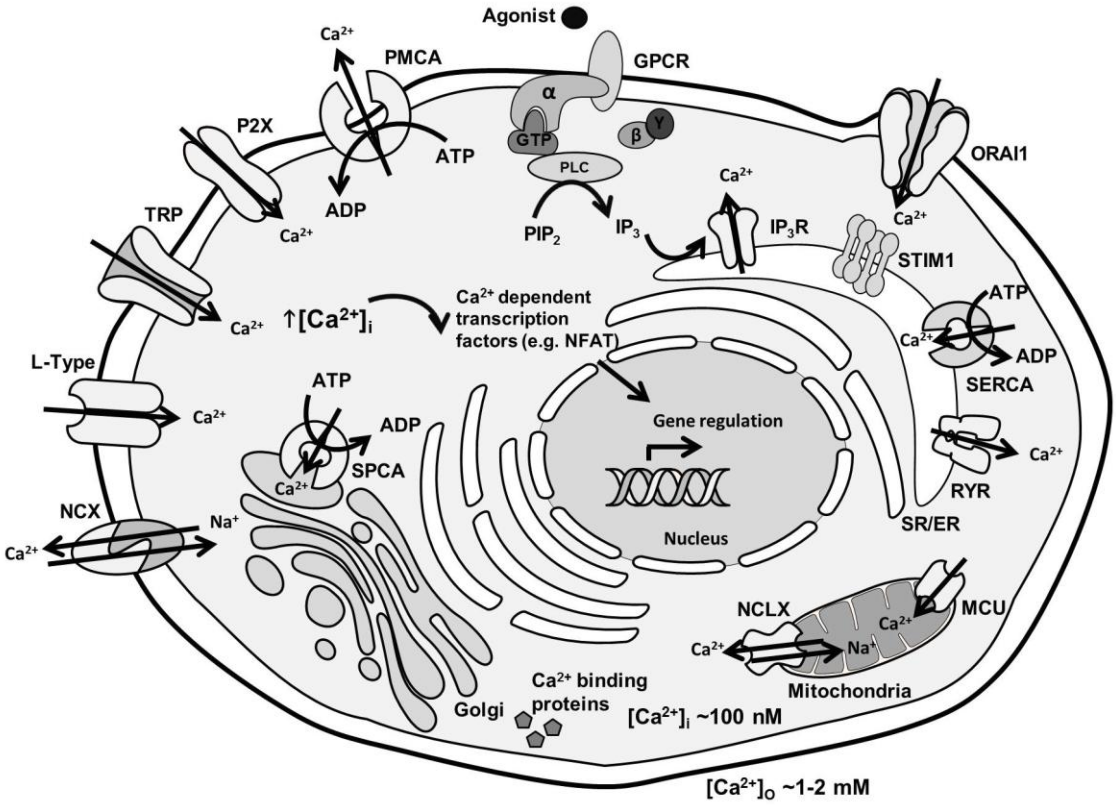

Fig. 1. Diagrammatic representation of major Ca^{2+} influx/efflux/release and resequestration pathways involved in the regulation of $[Ca^{2+}]_i$ homeostasis in mammalian cells and their associated proteins. Major Ca^{2+} influx pathways include those mediated by the transient receptor potential (TRP) family of Ca^{2+} permeable ion channels, voltage-gated Ca^{2+} channels (e.g. L-type), purinergic receptors (e.g. P2X), and the SOCE pathway components Orai1 and STIM1. Activation of plasma membrane localized G protein-coupled receptors (GPCRs) leads to generation of inositol triphosphate (IP_3) and subsequent stimulation of IP_3 receptors (IP_3Rs) located on the endoplasmic reticulum (ER), resulting in Ca^{2+} store release. ER localized ryanodine receptors (RYR) and mitochondrial Na^+/Ca^{2+} exchanger (NCLX) also regulate Ca^{2+} in organelles. The sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA), secretory pathway Ca^{2+} ATPase (SPCA), and mitochondrial uniporter (MCU) all sequester cytosolic Ca^{2+} into intracellular organelles, while plasma membrane Ca^{2+}-ATPases (PMCA) actively extrude Ca^{2+} from the cytosol into the extracellular space, and together with the Na^+/Ca^{2+} exchanger (NCX) play a role in restoring resting $[Ca^{2+}]_i$. Ca^{2+} signaling also regulates various Ca^{2+} dependent transcription factors (e.g. NFAT) and Ca^{2+} binding proteins (e.g. calmodulin). Adapted from references [8-10].

2. Remodeling of the calcium signal in disease

The development of Ca^{2+} sensitive indicators, such as the fluorescent dyes Fura-2 and Fluo-4, and genetically encoded Ca^{2+} indicators have been integral to our understanding and interpretation of intracellular calcium signaling by enabling quantitative analysis of Ca^{2+} in the cytoplasm and in subcellular organelles [11-16]. These tools have allowed a better understanding of how the nature of the calcium signal is remodeled in some diseases. A relatively well studied example of pathological remodeling of the calcium signal, reviewed in detail elsewhere [17-20], is that which occurs in smooth muscle cells as a consequence of vascular disease and injury, including pulmonary hypertension [21, 22], atherosclerosis [23, 24] and arterial restenosis following angioplasty [25, 26].

Calcium signaling in smooth muscle cells regulates numerous cellular processes including proliferation, contraction and gene transcription [27-30]. During vascular injury (through mechanical stress and/or growth factors/cytokine exposure), vascular smooth muscle cells can undergo phenotypic switching from cells that are largely quiescent and contractile, to those exhibiting a more synthetic and proliferative phenotype [18, 31, 32]. This phenotypic switching [31], is associated with corresponding changes in the *nature* of the calcium signal, for example a transition from voltage-gated Ca^{2+} entry pathways typical of contractile cells to one resembling store-operated and receptor-operated Ca^{2+} entry (SOCE) in proliferating cells [20, 33-35]. Kumar et al. demonstrated an example of such remodeling using an *in vivo* model of neointimal hyperplasia [25]. In this model, freshly isolated periadventitial cuff injured mouse carotid artery displayed increased $[Ca^{2+}]_i$ in response to reintroduction of Ca^{2+} following store depletion using the sarco/endoplasmic reticulum ATPase (SERCA) inhibitor thapsigargin, while K^+ induced depolarization failed to significantly increase $[Ca^{2+}]_i$ relative to uninjured arterial tissue [25]. These findings indicated a switch from a predominately

voltage-gated calcium entry pathway to one resembling SOCE following vascular injury. This change has been replicated in numerous *in vitro* models of vascular smooth muscle cell remodeling [33-37]. Changes in calcium signaling associated with neointimal hyperplasia are associated with increased expression of the transient receptor potential family member TRPC1, [25] a protein implicated in the SOCE pathway in some cells [38, 39]. Subsequent papers, relevant to this model, also identified roles for Orai1 and stromal interaction molecule 1 (STIM1) [33, 35, 40, 41], the canonical proteins involved in SOCE [38, 42-46]. These included the ability of Orai1 or STIM1 silencing to reduce neointima formation following balloon-injury of rat carotid artery [40, 47, 48].

Vascular smooth muscle injury clearly represents an example of dynamic remodeling of calcium signaling in disease and how characterization of these calcium signaling changes can lead to a better understanding of disease mechanisms and/or help identify potential therapeutic targets. In addition to pathologies involving vascular smooth muscle cell remodeling, deregulated calcium homeostasis has also been linked to remodeling of airway smooth muscle cells in asthma [49-51], and various other conditions, including those of the brain [52] such as Alzheimer's disease [53-56]. These studies have included the use of advanced assessment of calcium signaling, such as *in vivo* multiphoton fluorescence lifetime imaging microscopy of Ca^{2+} levels in astrocytes in a mouse model of Alzheimer's disease versus wild type/non-transgenic control animals [56].

Numerous studies have demonstrated altered expression of various components of the *"calcium signaling toolkit"* [1, 2] in cancer cell lines and in clinical samples (reviewed in [10, 57-61]). However, there have arguably been less studies comparing how the nature of the calcium signal is altered in cancer and/or changes with tumor progression, especially when

compared to other disease states (such as those described above). Although expression studies have identified specific Ca^{2+} channels and pumps as drug targets for various cancer types including ovarian [62], brain [63, 64], prostate [65, 66], breast [67, 68], and esophageal [69], calcium signaling could be altered in cancer progression through other changes, such as altered calcium channel or pump localization or activity via disrupted post-translational modification. Such changes would be better identified through assessment of the calcium signal rather than protein or mRNA levels. Indeed Ingueneau et al. reported that oxidized low-density-lipoprotein induced Ca^{2+} influx, and consequent apoptosis in vascular smooth muscle cells requires translocation of TRPC1 from an intracellular compartment to caveolae/caveolin-1 containing regions of the plasma membrane [70]. The cellular localization and activity of other TRP family members is also altered in response to various activation pathways in other cell types [71]. Post-translational protein modifications may also mask alterations in cellular calcium signaling if interpreted in the absence of functional studies. For example, Sundivakkam et al. showed that phosphorylation of STIM1 inhibits SOCE in endothelial cells, and is potentially involved in regulating blood vessel permeability responses [72]. Some studies have compared calcium signaling in cancer derived and relevant non-cancer derived cells and/or other models such as those involving cancer cell differentiation. This review will provide a summary of such studies and their significance. We will also describe some of the experimental constraints and challenges in characterizing calcium signaling changes associated with cancer progression, before discussing some of the experimental approaches that may allow these challenges to be addressed.

2.1 Remodeling of the calcium signal in tumor derived cells versus normal cells

The nature of the calcium signal plays an important role in regulating cellular functions [1, 3] including those defined as the *"hallmarks of cancer"* [59, 73]. One way to identify and

understand the possible remodeling of calcium homeostasis in some cancers is to compare the nature of the calcium signal in cells or cell lines derived from cancers with those derived from non-cancer tissue. Table 1 provides a summary of studies that have assessed differences in calcium homeostasis in tumor derived versus non-tumor derived cells. As discussed below, many of the changes between tumor and non-tumor derived cells are reflected in very specific changes in aspects of the calcium signal, such as the nature of Ca^{2+} influx or the rate of recovery of $[Ca^{2+}]_i$ after stimulation. Some of these changes are illustrated in Fig. 2.

Table 1

Examples of differences in Ca^{2+} homeostasis from studies comparing tumor versus non-tumor models.

Model studied[b]	Observed change in Ca^{2+} signaling in tumor model(s) relative to control(s)[c]	Potential consequence(s) of altered Ca^{2+} signaling	References
Human J82, RT24, T24 and 5637 bladder urothelial carcinoma cell lines vs. normal bladder urothelial cells from healthy human subjects	Absence of carbachol stimulated $[Ca^{2+}]_i$ increases	Possible alterations in cell adhesion	[74]
Human 5367 bladder urothelial carcinoma cell line vs. normal human urothelial primary cell lines from healthy controls	↓ Mechanically stimulated Ca^{2+} wave propagation	Altered intercellular communication via gap junctions	[75]
Human breast lobular infiltrating carcinoma derived endothelial cells vs. adult human dermal microvascular endothelial cells	↑ 4αPDD (a selective TRPV4 agonist) and arachidonic acid mediated $[Ca^{2+}]_i$ influx	Role in tumor angiogenesis and tumor derived endothelial cell migration	[76, 77]
Human SKBR3 breast cancer cell line vs. non-tumorigenic HBL100 mammary epithelial cell line	Remodeled SOCE and store depletion kinetics	Different contribution of calcium influx pathways and hence altered cellular responses to stimuli	[78]
Murine RAW 264.7 monocytic cell line treated with human MDA-MB-231 metastatic breast cancer cell line conditioned media vs. non-tumorigenic MCF-10A breast cell line conditioned media	Induction of sustained $[Ca^{2+}]_i$ oscillations following treatment of RAW 264.7 cells with MDA-MB-231 conditioned media	Potential role in osteoclast formation and bone metastases	[79]
Rat colonocytes from DMH procarcinogen treated animals	↓ Basal $[Ca^{2+}]_i$ in colonocytes from DMH treated rats	Reduced apoptosis	[80]

vs. colonocytes from vehicle treated animals			
Human KYSE-150 esophageal squamous cell carcinoma cell line vs. non-malignant HET-1A esophageal epithelial cell line	↑ SOCE and spontaneous Ca^{2+}_i oscillations	May promote cell proliferation, migration and invasion	[81]
Human U251 glioblastoma cell line, GBM1 and GBM8 primary glioblastoma multiforme cell lines vs. non-malignant human primary astrocytes	↑ SOCE	Promotion of cell invasion	[63]
Human SK-Mel-2, SK-Mel-24, C8161 and UACC257 metastatic melanoma cell lines vs. control HEMA-LP melanocyte cell line	↑ SOCE	Promotion of cell proliferation and migration	[82]
Rat pancreatic acinar carcinoma cells vs. normal acinar cells	↑ Rate of $[Ca^{2+}]_i$ recovery following stimulation by carbamylcholine and peptidergic agonist cholecystokinin octapeptide	Altered cellular responses to some stimuli and possible reduced apoptosis sensitivity via enhanced Ca^{2+} efflux	[83]
Human endothelial progenitor cells from renal cellular carcinoma patients vs. control endothelial progenitor cells	↑ SOCE	Enhanced proliferation and tubulogenesis	[84]
Human peripheral blood lymphocytes CLL patients vs. normal peripheral blood lymphocytes from healthy controls	↑ ATP stimulated $[Ca^{2+}]_i$ in some CLL patient samples.	Altered response to ATP effect	[85]
Human peripheral blood leukocytes from CML patients vs. normal peripheral blood leukocytes from healthy controls	↓ IP_3, ATP and ionomycin stimulated $[Ca^{2+}]_i$	Altered oxidative stress response	[86]
Human CEM and Jurkat malignant T cell lines vs. normal human peripheral blood T cells	↑ $[Ca^{2+}]_i$ in response to L-type Ca^{2+} channel activation by BAYK8644 and ionomycin stimulation	Altered sensitivity to apoptotic inducing agents and/or differential regulation of Ca^{2+} sensitive pathways	[87]

[a]Abbreviations: 4αPDD, 4α-Phorbal 12,13-didecanoate; ATP, adenosine triphosphate; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; DMH, 1,2-dimethylhydrazine dihydrochloride; IP_3, inositol 1,4,5-trisphosphate; SOCE, Store Operated Calcium Entry; TRPV4, transient receptor potential cation channel, subfamily V, member 4.

[b]Includes human and/or animal models of both solid and hematological cancers as well as those using conditioned media treatments.

[c]Arrows indicate either an increase (↑) or decrease (↓) in the nature of the Ca^{2+} signal in tumor model(s) relative to the non-tumor control(s).

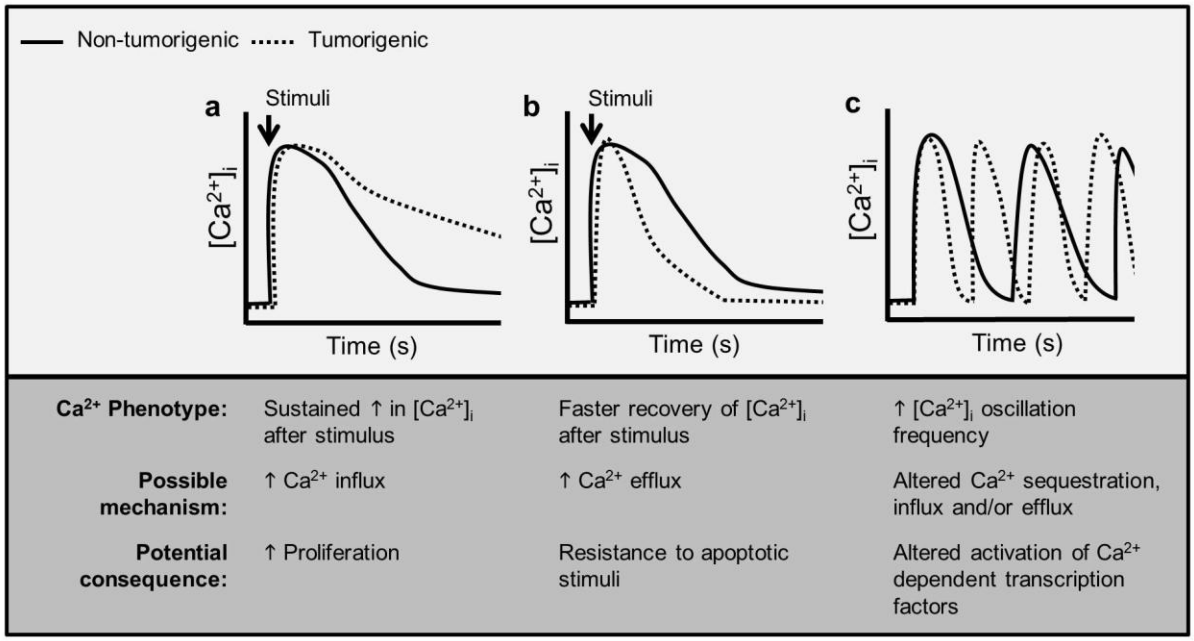

Fig. 2. Examples of hypothetical remodeling of the Ca^{2+} signal in tumorigenic versus non-tumorigenic cells. Each stylized Ca^{2+} trace depicts a remodeling of various aspects of intracellular Ca^{2+} signaling pathways, including (a) Ca^{2+} influx pathways, (b) Ca^{2+} efflux pathways, and (c) intracellular Ca^{2+} oscillatory behavior.

Since the identification of the key molecular components of SOCE, namely STIM1 and Orai1, there has been a keen interest in determining the role of this calcium influx pathway in pathophysiology [88-91]. Indeed, altered expression of STIM1 and/or Orai1 has been reported in various cancer types including breast [67], cervical [92], and esophageal [81]. Prior to the identification of the STIM and Orai proteins, Baldi et al. conducted studies looking into the nature and remodeling of capacitative Ca^{2+} entry (a term used synonymously with SOCE) in the tumorigenic (luminal human epidermal growth factor receptor 2 positive) SKBR3 cell line, and HBL100, which they used to represent non-tumorigenic breast epithelial cells [78]. While both cell lines demonstrated classic SOCE in response to thapsigargin mediated store depletion in the absence of extracellular Ca^{2+}, the nature of store release and Ca^{2+} re-entry (influx) noticeably differed between the two cell lines. While the

peak amplitude and initial rate of calcium influx was similar in both cell lines, Baldi et al. reported a more sustained and slower return to baseline $[Ca^{2+}]_i$ levels following SOCE in SKBR3 cells. Also, the amplitude of the initial peak representing store depletion appeared to be higher in HLB100 cells. The contribution of SOCE and the potential role of other Ca^{2+} entry pathways were also assessed using various surrogate divalent cations (Ba^{2+}, Mn^{2+} and Sr^{2+}), which demonstrate different permeabilities, and the lanthanide Gd^{3+}, which is known to block SOCE at low concentrations. From their studies, Baldi et al. characterized two main calcium influx pathways in both cell lines; however, their relative contributions to SOCE differed between the tumorigenic SKBR3 and non-tumorigenic HLB100 cell lines. These findings raised the question of whether differences in the nature of SOCE between both cell lines could be a function of tumorigenic remodeling, or merely due to other differences between these cell lines [78]. Interestingly, later studies by McAndrew et al. quantified the expression levels of Orai1, STIM1 and STIM2 in a panel of non-malignant and breast cancer cell lines [67]. Of the six breast cancer cell lines assessed, SKBR3 cells expressed the lowest levels of Orai1 mRNA (relative to the non-tumorigenic cell line 184A1), and an altered STIM1/STIM2 ratio compared to other tumorigenic cell lines. Unfortunately, while mRNA expression levels of the STIM and Orai family members was comprehensively characterized, a comparison of calcium signaling dynamics in all cell lines was not performed. Such a study may provide insight into the functional consequences of altered Orai1, STIM1 and STIM2 expression not only between non-tumorigenic and tumorigenic cell lines, but also between breast cancer cell lines representing different molecular subtypes.

Investigation into the contribution of SOCE, this time in glial cells, revealed a remodeling of this Ca^{2+} entry pathway in the form of a two-fold increase in the amplitude of SOCE in cultured human primary malignant glioblastoma multiforme cells relative to a non-malignant

human primary astrocyte control [63]. Further investigation revealed an increase in Orai1 mRNA levels in only two of the three glioblastoma multiforme cell lines assessed, despite SOCE being increased in all three malignant cell lines. This further supports the importance of comparing functional calcium signaling in cancer and non-cancer control cells in addition to assessment of gene expression. Enhanced SOCE indicated by significantly increased $[Ca^{2+}]_i$ peak amplitude following store depletion, and corresponding to increased Orai1 protein expression, was also shown in four metastatic melanoma cell lines relative to a control melanocyte cell line [82]. Zhu et al. recently identified a remodeling of the SOCE pathway in an esophageal squamous cell carcinoma derived cell line KYSE-150, relative to a non-tumorigenic esophageal epithelial cell line HET-1A [81]. In addition to assessing SOCE specifically, the authors also evaluated differences in global calcium signaling using live cell imaging in the absence of a specific stimulus. Esophageal squamous cell carcinoma cells displayed a significantly higher degree of spontaneous intracellular Ca^{2+} oscillations compared to normal cells, 76% versus 26%, respectively [81]. These oscillations could be inhibited by pharmacologically mediated SOCE blockade with SKF96365 and Orai1 silencing [81]. This is significant because of the importance of the nature of calcium oscillations in the regulation of the transcription factors that regulate genes important in cell proliferation and/or migration [93-96].

Altered expression of other Ca^{2+} entry pathways, in particular certain members of the transient receptor potential (TRP) family of Ca^{2+} permeable ion channels has also been identified in various cancer types including breast [68, 97, 98], prostate [61, 99], ovarian [62], esophageal [69], and brain [64] (reviewed in [61, 100]). Studies comparing cancer and non-cancer derived cells have also shown changes in calcium signaling mediated by some members of the TRP channel family. Early studies by Fiorio Pla et al. investigating

mechanisms of endothelial cell migration, a process important in angiogenesis in tumors, showed that the second messenger arachidonic acid induced a significantly higher Ca^{2+} response in breast tumor derived endothelial cells relative to normal dermal endothelial cells [76]. This difference appears to be mediated by the TRP channel family member TRPV4, since arachidonic acid and the TRPV4 agonist 4 α PDD stimulated Ca^{2+} entry in tumor derived endothelial cells is attenuated by TRPV4 silencing [77]. Another TRP family member showing altered activity in certain cancer types is the cold stimulus activated TRPM8, which mediated significantly greater current density (pA/pF) (determined via whole cell patch clamp) in an androgen sensitive primary prostate cancer cell line relative to normal primary prostate epithelial cells in response to icilin stimulation [66].

It is now well recognized that tumor cells do not exist in isolation but are rather part of a larger microenvironment comprising multiple cell types, including immune, endothelial and other supporting stromal cells, which together act to maintain and promote tumor growth and progression (reviewed in [101-104]). There is increasing evidence to suggest that the recruitment of endothelial progenitor cells from the bone marrow plays an important role in tumoral neoangiogenesis (reviewed in [105, 106]), which is essential for sustaining tumor growth and facilitating metastatic spread [107]. Remodeling of calcium signaling was recently identified in endothelial progenitor cells derived from patients with renal cell carcinoma relative to healthy controls [84]. Lodola et al. showed that the amplitude of SOCE was significantly higher in endothelial progenitor cells derived from renal cell carcinoma patients compared to those derived from healthy controls. This finding was supported by a corresponding increase in the transcript and protein levels of STIM1, Orai1 and TRPC1 in endothelial progenitor cells derived from renal cell carcinoma patients. Through a series of experiments, the authors provided evidence to support the hypothesis that the increase in

SOCE in endothelial progenitor cells derived from renal cell carcinoma patients increased proliferation and tubulogenesis, processes important in endothelial progenitor cell mediated neoangiogenesis [84].

Another example of the importance of calcium signaling in cells of the tumor microenvironment was shown by Tiedemann et al. when they demonstrated significantly increased intracellular Ca^{2+} oscillatory behavior in osteoclast (bone resorbing) precursor cells following stimulation with conditioned media from tumorigenic breast cancer cells, but not non-tumorigenic breast epithelial cells [79]. This increase in conditioned media stimulated Ca^{2+} oscillations was associated with a significant increase in osteoclast number and size, an effect that was effectively inhibited by treatment with the Ca^{2+} chelator BAPTA (1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid). This further supported a role for Ca^{2+} in the process of breast cancer cell induced osteoclastogenesis [79]. This finding is significant as increased osteoclast activity mediated by metastatic breast cancer cells in the bone microenvironment can lead to bone destruction, increased fracture risk and pain in cancer patients [79, 108].

The studies described above provide evidence for the value of comparing calcium signaling in tumor-derived cells with those derived from non-diseased tissue. However, in some cases experimental limitations make it difficult to determine how well these changes in calcium signaling actually reflect the changes associated with tumor development. In section 4 we will describe some of the methodological advancements that may address these limitations.

3. Differentiated cell lines as a model to study remodeling of calcium signaling in cancer cells

In order to overcome some of the difficulties associated with matching cancer cells to suitable normal control cells, a number of researchers have turned to tumorigenic cell lines in which a differentiated phenotype can be induced as a surrogate for 'normal' or at least less tumorigenic cells. The use of specific cell culture methods and/or stimulation with selected growth factors/pharmacological agents to induce a different cellular phenotype is not unique to cancer cell line models. Researchers investigating methods of culturing primary vascular smooth muscle cells observed that under certain culture conditions, smooth muscle cells can transition between a differentiated contractile and typically quiescent phenotype to a dedifferentiated and synthetic phenotype, characterized by a loss of contractile response, altered morphology and increased proliferative and migratory potential [109-114]. The phenotypic switching of vascular smooth muscle cells in culture is associated with changes in the nature of the calcium signal [33-35]. These changes include an increase in Ca^{2+} influx via SOCE. Any *in vitro* model cannot be expected to fully recapitulate conditions and remodeling events that occur *in vivo*. However, similar calcium signaling remodeling has been reported using an *in vivo* model of vascular injury [25]. Such agreement between *in vivo* and *in vitro* models of smooth muscle injury suggests that the differentiation or dedifferentiation of cell lines may be a useful approach to study the remodeling of calcium signaling in other disease states, including cancer. As described below, alterations in calcium signaling with differentiation have been reported in a variety of cancer cell lines including those of the breast, colon and lung.

In a similar way to groups culturing vascular smooth muscle cells to induce a 'switch' from a contractile to a synthetic phenotype [33, 35, 41], Bidaux et al. developed a model of prostate

epithelial cell dedifferentiation to aid in characterizing expression and activity of the TRP family member, TRPM8, during prostate cancer progression [66]. The Na^+ and Ca^{2+} permeable TRPM8 channel has been identified as a potential diagnostic/prognostic marker in prostate cancer [99, 115-118]. Prolonged culture of primary prostate epithelial cells led to a less differentiated phenotype corresponding with a loss of androgen receptor expression, which was associated with decreased TRPM8 activity in response to stimulation with the TRPM8 activator menthol. Confocal imaging of TRPM8 localization demonstrated a loss of TRPM8 from the plasma membrane as a consequence of dedifferentiation [66]. Other changes in Ca^{2+} influx pathways as a consequence of differentiation are reflected by the effects of the differentiating agent 9-*cis* retinoic acid on N- and S-type neuroblastoma cells derived from the SH-SY5Y neuroblastoma cell line [119]. Differentiation of the more malignant N-type cells is associated with the down regulation of SOCE, leading the authors to propose the utility of therapeutically targeting SOCE as a means for promoting neuroblastoma cell differentiation [119]. In the A549 lung cancer cell line, differentiation via all-*trans*-retinoic acid is associated with enhanced Ca^{2+} influx following trypsin-mediated Ca^{2+} store depletion [120], demonstrating that the down regulation of Ca^{2+} influx is not a ubiquitous feature of the differentiation of cancer cell lines.

Pharmacologically induced differentiation of cancer cells using agents such as those acting on histone deacetylase, including short chain fatty acids and their derivatives, and the protein kinase C activator phorbal 12-myristate 13-acetate (PMA) is another method used to study altered calcium signaling and homeostasis in cancer cells [121-125]. Recently, Varga et al. showed that short chain fatty acid and/or PMA induced differentiation of the MCF-7 breast cancer cell line correlates with an increase in plasma membrane Ca^{2+} ATPase isoform 4b (PMCA4b) protein and mRNA expression [121]. PMCA4 was also detected in normal breast

tissue sections following immunohistochemical staining [121]. These findings are in agreement with an earlier study, which showed decreased PMCA4 mRNA expression in a panel of breast cancer cell lines relative to non-tumorigenic breast epithelial cell lines [126]. In order to further investigate the effect of differentiation on the nature of the calcium signal, Varga et al. developed an MCF-7 cell line stably expressing the genetically encoded Ca^{2+} indicator GCaMP2 [121]. Valerate and/or the combination of valerate and PMA induced differentiation resulted in a remodeling of the calcium signal characterized by reduced peak $[Ca^{2+}]_i$ following adenosine triphosphate (ATP) mediated store depletion, as well as a much faster recovery of $[Ca^{2+}]_i$ to baseline, relative to undifferentiated control cells. Peak $[Ca^{2+}]_i$ was also greatly reduced in differentiated cells following calcium ionophore treatment. The authors proposed a role for PMCA4b in maintaining Ca^{2+} homeostasis in normal mammary epithelial cell physiology, a function that may be lost as cells progress to a tumorigenic phenotype [121].

Breast cancer cells are not the only cancer cell type demonstrating a differentiation induced remodeling of the calcium signal corresponding to altered PMCA expression and function. Indeed, Ribiczey et al. showed increased PMCA4b expression following short chain fatty acid induced differentiation of a gastric and colon carcinoma cell line, corresponding to a two to three fold increase in PMCA transport activity in isolated microsomal membrane vesicles [123]. Pharmacologically induced differentiation also resulted in increased PMCA2, PMCA3 and PMCA4 isoform expression in a human neuroblastoma cell line, which is associated with a faster recovery rate of $[Ca^{2+}]_i$ to baseline after depolarization [127].

In addition to plasma membrane localized Ca^{2+} efflux pathways, evidence of remodeling of intraorganellar Ca^{2+} sequestration pathways and homeostasis, specifically those involving the

sarco/endoplasmic reticulum Ca^{2+} ATPases (SERCAs), has also been demonstrated following differentiation of cancer cells. SERCAs play an important role in the regulation of endoplasmic reticulum Ca^{2+} homeostasis and altered expression of the SERCA isoforms, particularly SERCA3, has been identified in various cancer subtypes [124, 128-130]. Arbabian et al. demonstrated an increase in SERCA3 protein expression upon short chain fatty acid induced differentiation in a panel of lung adenocarcinoma cell lines [122]. Using A549 lung carcinoma derived cell lines stably expressing the genetically encoded calcium indicator GCaMP2, the authors reported significantly decreased Ca^{2+} store release induced by the SERCA inhibitor thapsigargin in phenylbutyrate differentiated cells relative to untreated control, whereas subsequent SOCE was essentially unaltered [122]. This finding was partially attributed to increased levels of the lower Ca^{2+} affinity SERCA3 isoform relative to that of the higher Ca^{2+} affinity SERCA2 isoform in differentiated cells [122]. Increased SERCA3 expression and altered Ca^{2+} homeostasis was also shown in the gastric carcinoma cell line, KATO-III, following butyrate induced differentiation [124]. Differentiation of KATO-III cells was associated with increased basal cytosolic Ca^{2+} relative to non-differentiated cells, and decreased thapsigargin induced endoplasmic reticulum Ca^{2+} store release [124], as was reported by Arbabian et al. in differentiated A549 cells [122].

The findings from the various models of differentiation described above provide further evidence of a remodeling of calcium signaling during the process of tumorigenesis. They also provide a rationale for future *in vitro* investigations using matched normal and tumorigenic primary and/or immortalized cell lines, as well as carefully designed *in vivo* experiments, to further study remodeling of the calcium signal in the context of cancer.

4. Modulation of the calcium signal during processes associated with invasion and metastasis

The transformation from benign to malignant disease is a leading cause of cancer related death [131, 132], and therapies targeted towards inhibiting processes important in the invasion-metastasis cascade are a major focus of current cancer research [131-136]. Therefore understanding the remodeling of the calcium signal in invasion and metastasis could aid in the identification of novel therapeutic targets. Recently, researchers have used various models to replicate processes important in invasion and metastasis in order to help define changes in calcium signaling that may be important in tumor progression.

In order for cancer cells to successfully metastasize they must first acquire the ability to invade and migrate into their surrounding microenvironment and local vasculature before disseminating to a distant site [137-140]. A functional role for Ca^{2+} in the directional migration of lung fibroblasts was elegantly demonstrated using real time confocal Ca^{2+} imaging, where highly organized Ca^{2+} signals known as "calcium flickers", which are tightly regulated in both space and time were visualized in response to a chemotactic agent [141]. The stretch activated Ca^{2+} permeable TRP family member, TRPM7, together with inositol 1,4,5-triphosphate receptor, type 2 (IP_3R2) stimulated Ca^{2+} release were implicated in mediating these calcium flickers. Subsequent studies have shown a role for TRPM7 in the migration of both nasopharyngeal [142] and pancreatic carcinoma cell lines [143]. For a detailed review of the role of calcium in cancer metastasis refer to [59].

The SOCE pathway appears to play an important role in migration and invasion in various cancer cell types [63, 81, 82, 144-146]. Indeed, evidence of a role for Orai1 and/or STIM1 in cell migration and invasion has been shown both *in vitro* and *in vivo* [81, 82, 92, 144].

Recently, Umemura et al. showed pharmacological inhibition of SOCE and/or gene silencing of either STIM1 or Orai1 significantly decreased melanoma cell line migration *in vitro*, and the formation of lung metastasis *in vivo* [82]. Some studies have also provided important evidence that some cancer cell lines are more sensitive to silencing of SOCE components than their appropriate non-malignant cell line controls. Indeed, Motiani et al. showed that the silencing of STIM1 and Orai1 significantly reduces serum stimulated invasion of glioblastoma cells relative to normal human primary astrocytes [63].

Vascularization of tumoral tissues via induction of angiogenesis represents one of the original cancer hallmarks [73] and intracellular calcium signaling plays an important role in regulating this process [147-149]. Using live cell imaging Fiorio Pla et al. showed that migrating breast tumor endothelial cells, located at the wounded edge of a scratched cell monolayer, responded with a significantly higher Ca^{2+} influx with TRPV4 activation compared to non-migrating endothelial cells located away from the wound edge [77]. These studies suggest that migrating endothelial cells, which are important in tumor angiogenesis, undergo a remodeling of their calcium signaling and this is associated with increased responsiveness to TRPV4 activators. This remodeling may be due to dynamic changes in TRPV4 localization, since arachidonic acid (an effector in the angiogenic growth factor signaling pathway) caused redistribution of TRPV4 to the plasma membrane in endothelial cells, a function related to remodeling of the actin cytoskeleton. TRPV4 gene silencing abolished the migratory response of breast tumor endothelial cells to arachidonic acid [77], providing further evidence of a role for TRPV4 mediated Ca^{2+} influx in arachidonic acid stimulated breast tumor endothelial cell migration.

The ability of polarized cancer epithelial cells to transition to a mesenchymal and migratory state via epithelial-mesenchymal transition (EMT), a process thought to play a role in cancer metastasis [150-152], has recently been linked to calcium signaling [153]. Davis et al. demonstrated that mechanical wounding of an MDA-MB-468 breast cancer epithelial cell monolayer, a process shown to induce expression of the EMT marker vimentin in other tumorigenic and non-tumorigenic breast epithelial cell lines [154, 155], resulted in the propagation of a calcium wave from the site of wounding [153]. In addition, treatment of MDA-MB-468 breast cancer cells with exogenous epidermal growth factor (EGF) (another inducer of EMT in this model) also resulted in increases in cytosolic free Ca^{2+}. Although the buffering of intracellular Ca^{2+} inhibited EMT induction by EGF, the nature of the calcium signal was critical to the ability of agents to induce EMT, and the Ca^{2+} permeable ion channel TRPM7 was identified as a key regulator of EGF-induced EMT in MDA-MB-468 breast cancer cells [153]. Further remodeling of the calcium signal was also demonstrated in this model of EMT, whereby cells induced to undergo EMT exhibited decreased Ca^{2+} influx induced by the purinergic receptor agonist ATP and the protease activated receptor 2 activator trypsin, as well as reduced SOCE [156]. In addition, cells in the more invasive mesenchymal state displayed significantly faster recovery of $[Ca^{2+}]_i$ after ATP stimulation [157]. Hence, EMT at least in this breast cancer cell line model is associated with a remodeling of the calcium signal [156]. A different remodeling of Ca^{2+} signaling as a consequence of EMT has been reported by Hu et al., who used TGF β to induce EMT in MCF-7 breast cancer cells, where EMT was associated with an increase in SOCE [158]. Further studies in these and others models of changes implicated in metastasis are required to fully understand how Ca^{2+} signaling may be altered in cells undergoing changes associated with the acquisition of a more invasive phenotype.

5. Novel methods for studying changes in the nature of the calcium signal in cancer

Whilst valuable in contributing to our understanding of the remodeling of the nature of the calcium signal in tumorigenesis, findings from studies such as those presented above can suffer limitations by virtue of the low throughput nature of the methods used. Methods often restrict the number of tumorigenic and normal (or non-tumorigenic) cell lines that can be compared. This may be particularly important in research using breast cancer cell lines, which are commonly classified according to molecular subtype [159-162] and represent an example where the use of multiple controls, both tumorigenic and non-tumorigenic, is often desirable in order to represent the heterogeneity seen in breast cancer [163-165]. This limitation can be partially addressed through use of high throughput devices, such as the fluorometric imaging plate reader (FLIPR®, Molecular Devices), which could enable the simultaneous analysis of multiple cell lines in microplates of 96, 384 or 1536 wells [166]. Although such instrumentation has been used to assess Ca^{2+} signals in breast cancer cells [67, 68, 153, 156, 157], their potential in fully characterizing many tumorigenic and non-tumorigenic cell lines has not been fully utilized.

Development of the Ca^{2+} sensitive fluorescent dyes (e.g. Fura-2 and Fluo-4) has been integral to our current understanding of intracellular calcium signaling in both physiology and pathophysiology. However, as discussed in section 2.1, there is an increasing interest in the remodeling of the calcium signal as a consequence of the interaction between tumor cells and cells of their microenvironment. The assessment of calcium signaling *in vivo* using advanced imaging techniques such as multi-photon microscopy is increasing, and enables investigation of calcium signaling in the context of the cellular microenvironment [56, 167-169]. While the development of the acetoxymethyl ester forms of the fluorescent dyes (e.g. Fura-2/AM), has provided a relatively non-invasive means of measuring intracellular calcium signaling in

living cells [170], their ability to become sequestered into Ca^{2+} containing intracellular organelles and/or extruded by multidrug transporters, including MDR1 (also known as P-glycoprotein), [171-173] (up-regulated in many cancer cells [174]) can limit their utility in long term studies of calcium signaling *in vivo*. The range of genetically encoded Ca^{2+} indicators now available (reviewed in [175-177]), which can be stably transfected into living cells, allows the measurement of calcium events over extended periods [176]. *In vivo* Ca^{2+} imaging has provided enormous insight into the role of calcium signaling in living systems (reviewed in [178]). Whilst this method is predominately currently used to study calcium signaling in neuronal networks [179-181], the potential exists for these tools to provide insight into the remodeling of the calcium signal during tumorigenic processes such as invasion and metastasis *in vivo*.

5. Conclusion

While expressional changes in proteins responsible for regulating intracellular calcium signaling, such as channels, pumps and exchangers, have been characterized in various cancer cell lines and tissues, specific alterations in the *nature* of the calcium signal as a cause and/or consequence of tumorigenesis are less well described. Remodeling of the calcium signal is a feature of various disease states, and has been well characterized in models of vascular injury and disease. The important role of calcium signaling in proliferation, cell death, and invasion and metastasis, represent multiple opportunities for targeting altered calcium signaling during the course of tumorigenesis. Models enabling comparison of the calcium signal between tumorigenic and non-tumorigenic phenotypes combined with the use of high throughput and/or advanced Ca^{2+} imaging methodologies may further aid in the

translation of altered calcium signaling assessment into the development of targeted cancer therapeutics.

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References

[1] M.J. Berridge, M.D. Bootman, H.L. Roderick, Calcium signalling: dynamics, homeostasis and remodelling, Nature reviews. Molecular cell biology, 4 (2003) 517-529.
[2] M.J. Berridge, P. Lipp, M.D. Bootman, The versatility and universality of calcium signalling, Nature reviews. Molecular cell biology, 1 (2000) 11-21.
[3] D.E. Clapham, Calcium signaling, Cell, 131 (2007) 1047-1058.
[4] K. Machaca, Ca(2+) signaling, genes and the cell cycle, Cell calcium, 49 (2011) 323-330.
[5] J. Parkash, K. Asotra, Calcium wave signaling in cancer cells, Life sciences, 87 (2010) 587-595.
[6] L. Missiaen, W. Robberecht, L. van den Bosch, G. Callewaert, J.B. Parys, F. Wuytack, L. Raeymaekers, B. Nilius, J. Eggermont, H. De Smedt, Abnormal intracellular ca(2+)homeostasis and disease, Cell calcium, 28 (2000) 1-21.
[7] H.L. Roderick, S.J. Cook, Ca2+ signalling checkpoints in cancer: remodelling Ca2+ for cancer cell proliferation and survival, Nature reviews. Cancer, 8 (2008) 361-375.
[8] E. Carafoli, The calcium-signalling saga: tap water and protein crystals, Nat Rev Mol Cell Biol, 4 (2003) 326-332.
[9] R.S. Lewis, The molecular choreography of a store-operated calcium channel, Nature, 446 (2007) 284-287.
[10] I. Azimi, S.J. Roberts-Thomson, G.R. Monteith, Calcium influx pathways in breast cancer: opportunities for pharmacological intervention, British journal of pharmacology, 171 (2014) 945-960.
[11] R.Y. Tsien, T.J. Rink, M. Poenie, Measurement of cytosolic free Ca2+ in individual small cells using fluorescence microscopy with dual excitation wavelengths, Cell calcium, 6 (1985) 145-157.
[12] G. Grynkiewicz, M. Poenie, R.Y. Tsien, A new generation of Ca2+ indicators with greatly improved fluorescence properties, The Journal of biological chemistry, 260 (1985) 3440-3450.
[13] A. Minta, J.P. Kao, R.Y. Tsien, Fluorescent indicators for cytosolic calcium based on rhodamine and fluorescein chromophores, The Journal of biological chemistry, 264 (1989) 8171-8178.

[14] P.H. Cobbold, T.J. Rink, Fluorescence and bioluminescence measurement of cytoplasmic free calcium, The Biochemical journal, 248 (1987) 313-328.
[15] J. Nakai, M. Ohkura, Probing calcium ions with biosensors, Biotechnology & genetic engineering reviews, 20 (2003) 3-21.
[16] M. Whitaker, Genetically encoded probes for measurement of intracellular calcium, Methods in cell biology, 99 (2010) 153-182.
[17] S.J. House, M. Potier, J. Bisaillon, H.A. Singer, M. Trebak, The non-excitable smooth muscle: calcium signaling and phenotypic switching during vascular disease, Pflugers Archiv : European journal of physiology, 456 (2008) 769-785.
[18] A. Marchand, A. Abi-Gerges, Y. Saliba, E. Merlet, A.M. Lompre, Calcium signaling in vascular smooth muscle cells: from physiology to pathology, Advances in experimental medicine and biology, 740 (2012) 795-810.
[19] M. Trebak, STIM/Orai signalling complexes in vascular smooth muscle, The Journal of physiology, 590 (2012) 4201-4208.
[20] V.V. Matchkov, O. Kudryavtseva, C. Aalkjaer, Intracellular Ca(2)(+) signalling and phenotype of vascular smooth muscle cells, Basic & clinical pharmacology & toxicology, 110 (2012) 42-48.
[21] R.A. Fernandez, P. Sundivakkam, K.A. Smith, A.S. Zeifman, A.R. Drennan, J.X. Yuan, Pathogenic role of store-operated and receptor-operated ca(2+) channels in pulmonary arterial hypertension, Journal of signal transduction, 2012 (2012) 951497.
[22] S. Zhang, H. Dong, L.J. Rubin, J.X. Yuan, Upregulation of Na+/Ca2+ exchanger contributes to the enhanced Ca2+ entry in pulmonary artery smooth muscle cells from patients with idiopathic pulmonary arterial hypertension, American journal of physiology. Cell physiology, 292 (2007) C2297-2305.
[23] D.K. Bowles, C.L. Heaps, J.R. Turk, K.K. Maddali, E.M. Price, Hypercholesterolemia inhibits L-type calcium current in coronary macro-, not microcirculation, Journal of applied physiology, 96 (2004) 2240-2248.
[24] T. Van Assche, P. Fransen, P.J. Guns, A.G. Herman, H. Bult, Altered Ca2+ handling of smooth muscle cells in aorta of apolipoprotein E-deficient mice before development of atherosclerotic lesions, Cell calcium, 41 (2007) 295-302.
[25] B. Kumar, K. Dreja, S.S. Shah, A. Cheong, S.Z. Xu, P. Sukumar, J. Naylor, A. Forte, M. Cipollaro, D. McHugh, P.A. Kingston, A.M. Heagerty, C.M. Munsch, A. Bergdahl, A. Hultgardh-Nilsson, M.F. Gomez, K.E. Porter, P. Hellstrand, D.J. Beech, Upregulated TRPC1 channel in vascular injury in vivo and its role in human neointimal hyperplasia, Circulation research, 98 (2006) 557-563.
[26] J.F. Quignard, M.C. Harricane, C. Menard, P. Lory, J. Nargeot, L. Capron, D. Mornet, S. Richard, Transient down-regulation of L-type Ca(2+) channel and dystrophin expression after balloon injury in rat aortic cells, Cardiovascular research, 49 (2001) 177-188.
[27] O. Kudryavtseva, C. Aalkjaer, V.V. Matchkov, Vascular smooth muscle cell phenotype is defined by Ca2+-dependent transcription factors, The FEBS journal, 280 (2013) 5488-5499.
[28] B.R. Wamhoff, D.K. Bowles, G.K. Owens, Excitation-transcription coupling in arterial smooth muscle, Circulation research, 98 (2006) 868-878.
[29] D. Bi, K. Toyama, V. Lemaitre, J. Takai, F. Fan, D.P. Jenkins, H. Wulff, D.D. Gutterman, F. Park, H. Miura, The intermediate conductance calcium-activated potassium channel KCa3.1 regulates vascular smooth muscle cell proliferation via controlling calcium-dependent signaling, The Journal of biological chemistry, 288 (2013) 15843-15853.
[30] R.W. Guo, L.X. Yang, M.Q. Li, X.H. Pan, B. Liu, Y.L. Deng, Stim1- and Orai1-mediated store-operated calcium entry is critical for angiotensin II-induced vascular smooth muscle cell proliferation, Cardiovascular research, 93 (2012) 360-370.

[31] G.K. Owens, M.S. Kumar, B.R. Wamhoff, Molecular regulation of vascular smooth muscle cell differentiation in development and disease, Physiological reviews, 84 (2004) 767-801.
[32] G.R. Campbell, J.H. Campbell, Smooth muscle phenotypic changes in arterial wall homeostasis: implications for the pathogenesis of atherosclerosis, Experimental and molecular pathology, 42 (1985) 139-162.
[33] R. Berra-Romani, A. Mazzocco-Spezzia, M.V. Pulina, V.A. Golovina, Ca2+ handling is altered when arterial myocytes progress from a contractile to a proliferative phenotype in culture, American journal of physiology. Cell physiology, 295 (2008) C779-790.
[34] E. Munoz, M. Hernandez-Morales, D. Sobradillo, A. Rocher, L. Nunez, C. Villalobos, Intracellular Ca(2+) remodeling during the phenotypic journey of human coronary smooth muscle cells, Cell calcium, 54 (2013) 375-385.
[35] M. Potier, J.C. Gonzalez, R.K. Motiani, I.F. Abdullaev, J.M. Bisaillon, H.A. Singer, M. Trebak, Evidence for STIM1- and Orai1-dependent store-operated calcium influx through ICRAC in vascular smooth muscle cells: role in proliferation and migration, FASEB journal : official publication of the Federation of American Societies for Experimental Biology, 23 (2009) 2425-2437.
[36] C.A. Emter, D.K. Bowles, Store-operated Ca(2+) entry is not essential for PDGF-BB induced phenotype modulation in rat aortic smooth muscle, Cell calcium, 48 (2010) 10-18.
[37] V.A. Golovina, Cell proliferation is associated with enhanced capacitative Ca(2+) entry in human arterial myocytes, The American journal of physiology, 277 (1999) C343-349.
[38] J.T. Smyth, S.Y. Hwang, T. Tomita, W.I. DeHaven, J.C. Mercer, J.W. Putney, Activation and regulation of store-operated calcium entry, Journal of cellular and molecular medicine, 14 (2010) 2337-2349.
[39] K.T. Cheng, H.L. Ong, X. Liu, I.S. Ambudkar, Contribution and regulation of TRPC channels in store-operated Ca2+ entry, Current topics in membranes, 71 (2013) 149-179.
[40] W. Zhang, K.E. Halligan, X. Zhang, J.M. Bisaillon, J.C. Gonzalez-Cobos, R.K. Motiani, G. Hu, P.A. Vincent, J. Zhou, M. Barroso, H.A. Singer, K. Matrougui, M. Trebak, Orai1-mediated I (CRAC) is essential for neointima formation after vascular injury, Circulation research, 109 (2011) 534-542.
[41] J.M. Bisaillon, R.K. Motiani, J.C. Gonzalez-Cobos, M. Potier, K.E. Halligan, W.F. Alzawahra, M. Barroso, H.A. Singer, D. Jourd'heuil, M. Trebak, Essential role for STIM1/Orai1-mediated calcium influx in PDGF-induced smooth muscle migration, American journal of physiology. Cell physiology, 298 (2010) C993-1005.
[42] M.D. Cahalan, STIMulating store-operated Ca(2+) entry, Nature cell biology, 11 (2009) 669-677.
[43] S.L. Zhang, Y. Yu, J. Roos, J.A. Kozak, T.J. Deerinck, M.H. Ellisman, K.A. Stauderman, M.D. Cahalan, STIM1 is a Ca2+ sensor that activates CRAC channels and migrates from the Ca2+ store to the plasma membrane, Nature, 437 (2005) 902-905.
[44] M. Prakriya, S. Feske, Y. Gwack, S. Srikanth, A. Rao, P.G. Hogan, Orai1 is an essential pore subunit of the CRAC channel, Nature, 443 (2006) 230-233.
[45] J. Soboloff, M.A. Spassova, X.D. Tang, T. Hewavitharana, W. Xu, D.L. Gill, Orai1 and STIM reconstitute store-operated calcium channel function, The Journal of biological chemistry, 281 (2006) 20661-20665.
[46] M. Potier, M. Trebak, New developments in the signaling mechanisms of the store-operated calcium entry pathway, Pflugers Archiv : European journal of physiology, 457 (2008) 405-415.
[47] R.W. Guo, H. Wang, P. Gao, M.Q. Li, C.Y. Zeng, Y. Yu, J.F. Chen, M.B. Song, Y.K. Shi, L. Huang, An essential role for stromal interaction molecule 1 in neointima formation following arterial injury, Cardiovascular research, 81 (2009) 660-668.

[48] F.C. Aubart, Y. Sassi, A. Coulombe, N. Mougenot, C. Vrignaud, P. Leprince, P. Lechat, A.M. Lompre, J.S. Hulot, RNA interference targeting STIM1 suppresses vascular smooth muscle cell proliferation and neointima formation in the rat, Molecular therapy : the journal of the American Society of Gene Therapy, 17 (2009) 455-462.
[49] K. Mahn, S.J. Hirst, S. Ying, M.R. Holt, P. Lavender, O.O. Ojo, L. Siew, D.E. Simcock, C.G. McVicker, V. Kanabar, V.A. Snetkov, B.J. O'Connor, C. Karner, D.J. Cousins, P. Macedo, K.F. Chung, C.J. Corrigan, J.P. Ward, T.H. Lee, Diminished sarco/endoplasmic reticulum Ca2+ ATPase (SERCA) expression contributes to airway remodelling in bronchial asthma, Proceedings of the National Academy of Sciences of the United States of America, 106 (2009) 10775-10780.
[50] A.M. Spinelli, J.C. Gonzalez-Cobos, X. Zhang, R.K. Motiani, S. Rowan, W. Zhang, J. Garrett, P.A. Vincent, K. Matrougui, H.A. Singer, M. Trebak, Airway smooth muscle STIM1 and Orai1 are upregulated in asthmatic mice and mediate PDGF-activated SOCE, CRAC currents, proliferation, and migration, Pflugers Archiv : European journal of physiology, 464 (2012) 481-492.
[51] K. Mahn, O.O. Ojo, G. Chadwick, P.I. Aaronson, J.P. Ward, T.H. Lee, Ca(2+) homeostasis and structural and functional remodelling of airway smooth muscle in asthma, Thorax, 65 (2010) 547-552.
[52] M. Brini, T. Cali, D. Ottolini, E. Carafoli, Neuronal calcium signaling: function and dysfunction, Cellular and molecular life sciences : CMLS, 71 (2014) 2787-2814.
[53] D.H. Small, Dysregulation of calcium homeostasis in Alzheimer's disease, Neurochemical research, 34 (2009) 1824-1829.
[54] C. Peers, I.F. Smith, J.P. Boyle, H.A. Pearson, Remodelling of Ca2+ homeostasis in type I cortical astrocytes by hypoxia: evidence for association with Alzheimer's disease, Biological chemistry, 385 (2004) 285-289.
[55] J.R. Lopez, A. Lyckman, S. Oddo, F.M. Laferla, H.W. Querfurth, A. Shtifman, Increased intraneuronal resting [Ca2+] in adult Alzheimer's disease mice, Journal of neurochemistry, 105 (2008) 262-271.
[56] K.V. Kuchibhotla, C.R. Lattarulo, B.T. Hyman, B.J. Bacskai, Synchronous hyperactivity and intercellular calcium waves in astrocytes in Alzheimer mice, Science, 323 (2009) 1211-1215.
[57] J.M. Lee, F.M. Davis, S.J. Roberts-Thomson, G.R. Monteith, Ion channels and transporters in cancer. 4. Remodeling of Ca(2+) signaling in tumorigenesis: role of Ca(2+) transport, American journal of physiology. Cell physiology, 301 (2011) C969-976.
[58] G.R. Monteith, F.M. Davis, S.J. Roberts-Thomson, Calcium channels and pumps in cancer: changes and consequences, The Journal of biological chemistry, 287 (2012) 31666-31673.
[59] N. Prevarskaya, R. Skryma, Y. Shuba, Calcium in tumour metastasis: new roles for known actors, Nature reviews. Cancer, 11 (2011) 609-618.
[60] N. Prevarskaya, R. Skryma, Y. Shuba, Targeting Ca(2+) transport in cancer: close reality or long perspective?, Expert opinion on therapeutic targets, (2013).
[61] N. Prevarskaya, L. Zhang, G. Barritt, TRP channels in cancer, Biochimica et biophysica acta, 1772 (2007) 937-946.
[62] S.L. Yang, Q. Cao, K.C. Zhou, Y.J. Feng, Y.Z. Wang, Transient receptor potential channel C3 contributes to the progression of human ovarian cancer, Oncogene, 28 (2009) 1320-1328.
[63] R.K. Motiani, M.C. Hyzinski-Garcia, X. Zhang, M.M. Henkel, I.F. Abdullaev, Y.H. Kuo, K. Matrougui, A.A. Mongin, M. Trebak, STIM1 and Orai1 mediate CRAC channel activity and are essential for human glioblastoma invasion, Pflugers Archiv : European journal of physiology, 465 (2013) 1249-1260.

[64] X. Ding, Z. He, K. Zhou, J. Cheng, H. Yao, D. Lu, R. Cai, Y. Jin, B. Dong, Y. Xu, Y. Wang, Essential role of TRPC6 channels in G2/M phase transition and development of human glioma, Journal of the National Cancer Institute, 102 (2010) 1052-1068.
[65] T. Fixemer, U. Wissenbach, V. Flockerzi, H. Bonkhoff, Expression of the Ca2+-selective cation channel TRPV6 in human prostate cancer: a novel prognostic marker for tumor progression, Oncogene, 22 (2003) 7858-7861.
[66] G. Bidaux, M. Flourakis, S. Thebault, A. Zholos, B. Beck, D. Gkika, M. Roudbaraki, J.L. Bonnal, B. Mauroy, Y. Shuba, R. Skryma, N. Prevarskaya, Prostate cell differentiation status determines transient receptor potential melastatin member 8 channel subcellular localization and function, The Journal of clinical investigation, 117 (2007) 1647-1657.
[67] D. McAndrew, D.M. Grice, A.A. Peters, F.M. Davis, T. Stewart, M. Rice, C.E. Smart, M.A. Brown, P.A. Kenny, S.J. Roberts-Thomson, G.R. Monteith, ORAI1-mediated calcium influx in lactation and in breast cancer, Molecular cancer therapeutics, 10 (2011) 448-460.
[68] A.A. Peters, P.T. Simpson, J.J. Bassett, J.M. Lee, L. Da Silva, L.E. Reid, S. Song, M.O. Parat, S.R. Lakhani, P.A. Kenny, S.J. Roberts-Thomson, G.R. Monteith, Calcium channel TRPV6 as a potential therapeutic target in estrogen receptor-negative breast cancer, Mol Cancer Ther, 11 (2012) 2158-2168.
[69] Y. Shi, X. Ding, Z.H. He, K.C. Zhou, Q. Wang, Y.Z. Wang, Critical role of TRPC6 channels in G2 phase transition and the development of human oesophageal cancer, Gut, 58 (2009) 1443-1450.
[70] C. Ingueneau, U.D. Huynh, B. Marcheix, A. Athias, P. Gambert, A. Negre-Salvayre, R. Salvayre, C. Vindis, TRPC1 is regulated by caveolin-1 and is involved in oxidized LDL-induced apoptosis of vascular smooth muscle cells, Journal of cellular and molecular medicine, 13 (2009) 1620-1631.
[71] V.J. Bezzerides, I.S. Ramsey, S. Kotecha, A. Greka, D.E. Clapham, Rapid vesicular translocation and insertion of TRP channels, Nature cell biology, 6 (2004) 709-720.
[72] P.C. Sundivakkam, V. Natarajan, A.B. Malik, C. Tiruppathi, Store-operated Ca2+ entry (SOCE) induced by protease-activated receptor-1 mediates STIM1 protein phosphorylation to inhibit SOCE in endothelial cells through AMP-activated protein kinase and p38beta mitogen-activated protein kinase, The Journal of biological chemistry, 288 (2013) 17030-17041.
[73] D. Hanahan, R.A. Weinberg, The hallmarks of cancer, Cell, 100 (2000) 57-70.
[74] B.T. Tully, M. Li, Y. Sun, J. Berkowitz, T.C. Chai, Defects in muscarinic receptor cell signaling in bladder urothelial cancer cell lines, Urology, 74 (2009) 467-473.
[75] P. Leinonen, V. Aaltonen, S. Koskela, P. Lehenkari, T. Korkiamaki, J. Peltonen, Impaired gap junction formation and intercellular calcium signaling in urinary bladder cancer cells can be improved by Go6976, Cell communication & adhesion, 14 (2007) 125-136.
[76] A. Fiorio Pla, T. Genova, E. Pupo, C. Tomatis, A. Genazzani, R. Zaninetti, L. Munaron, Multiple roles of protein kinase a in arachidonic acid-mediated Ca2+ entry and tumor-derived human endothelial cell migration, Molecular cancer research : MCR, 8 (2010) 1466-1476.
[77] A. Fiorio Pla, H.L. Ong, K.T. Cheng, A. Brossa, B. Bussolati, T. Lockwich, B. Paria, L. Munaron, I.S. Ambudkar, TRPV4 mediates tumor-derived endothelial cell migration via arachidonic acid-activated actin remodeling, Oncogene, 31 (2012) 200-212.
[78] C. Baldi, G. Vazquez, R. Boland, Capacitative calcium influx in human epithelial breast cancer and non-tumorigenic cells occurs through Ca2+ entry pathways with different permeabilities to divalent cations, Journal of cellular biochemistry, 88 (2003) 1265-1272.
[79] K. Tiedemann, O. Hussein, G. Sadvakassova, Y. Guo, P.M. Siegel, S.V. Komarova, Breast cancer-derived factors stimulate osteoclastogenesis through the Ca2+/protein kinase C and transforming growth factor-beta/MAPK signaling pathways, The Journal of biological chemistry, 284 (2009) 33662-33670.

[80] J. Kaur, S.N. Sanyal, Intracellular pH and calcium signaling as molecular targets of diclofenac-induced apoptosis against colon cancer, European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation, 20 (2011) 263-276.
[81] H. Zhu, H. Zhang, F. Jin, M. Fang, M. Huang, C.S. Yang, T. Chen, L. Fu, Z. Pan, Elevated Orai1 expression mediates tumor-promoting intracellular Ca2+ oscillations in human esophageal squamous cell carcinoma, Oncotarget, (2014).
[82] M. Umemura, E. Baljinnyam, S. Feske, M.S. De Lorenzo, L.H. Xie, X. Feng, K. Oda, A. Makino, T. Fujita, U. Yokoyama, M. Iwatsubo, S. Chen, J.S. Goydos, Y. Ishikawa, K. Iwatsubo, Store-operated Ca2+ entry (SOCE) regulates melanoma proliferation and cell migration, PloS one, 9 (2014) e89292.
[83] J.L. Chien, J.R. Warren, Free calcium and calmodulin levels in acinar carcinoma and normal acinar cells of rat pancreas, International journal of pancreatology : official journal of the International Association of Pancreatology, 3 (1988) 113-127.
[84] F. Lodola, U. Laforenza, E. Bonetti, D. Lim, S. Dragoni, C. Bottino, H.L. Ong, G. Guerra, C. Ganini, M. Massa, M. Manzoni, I.S. Ambudkar, A.A. Genazzani, V. Rosti, P. Pedrazzoli, F. Tanzi, F. Moccia, C. Porta, Store-operated Ca2+ entry is remodelled and controls in vitro angiogenesis in endothelial progenitor cells isolated from tumoral patients, PloS one, 7 (2012) e42541.
[85] J.S. Wiley, G.R. Dubyak, Extracellular adenosine triphosphate increases cation permeability of chronic lymphocytic leukemic lymphocytes, Blood, 73 (1989) 1316-1323.
[86] R. Ciarcia, D. d'Angelo, C. Pacilio, D. Pagnini, M. Galdiero, F. Fiorito, S. Damiano, E. Mattioli, C. Lucchetti, S. Florio, A. Giordano, Dysregulated calcium homeostasis and oxidative stress in chronic myeloid leukemia (CML) cells, Journal of cellular physiology, 224 (2010) 443-453.
[87] S. Baumann, S.C. Fas, M. Giaisi, W.W. Muller, A. Merling, K. Gulow, L. Edler, P.H. Krammer, M. Li-Weber, Wogonin preferentially kills malignant lymphocytes and suppresses T-cell tumor growth by inducing PLCgamma1- and Ca2+-dependent apoptosis, Blood, 111 (2008) 2354-2363.
[88] A. Berna-Erro, G.E. Woodard, J.A. Rosado, Orais and STIMs: physiological mechanisms and disease, Journal of cellular and molecular medicine, 16 (2012) 407-424.
[89] P.J. Shaw, S. Feske, Physiological and pathophysiological functions of SOCE in the immune system, Frontiers in bioscience, 4 (2012) 2253-2268.
[90] S.J. Roberts-Thomson, A.A. Peters, D.M. Grice, G.R. Monteith, ORAI-mediated calcium entry: mechanism and roles, diseases and pharmacology, Pharmacology & therapeutics, 127 (2010) 121-130.
[91] S. Feske, CRAC channelopathies, Pflugers Archiv : European journal of physiology, 460 (2010) 417-435.
[92] Y.F. Chen, W.T. Chiu, Y.T. Chen, P.Y. Lin, H.J. Huang, C.Y. Chou, H.C. Chang, M.J. Tang, M.R. Shen, Calcium store sensor stromal-interaction molecule 1-dependent signaling plays an important role in cervical cancer growth, migration, and angiogenesis, Proceedings of the National Academy of Sciences of the United States of America, 108 (2011) 15225-15230.
[93] R.E. Dolmetsch, K. Xu, R.S. Lewis, Calcium oscillations increase the efficiency and specificity of gene expression, Nature, 392 (1998) 933-936.
[94] W. Li, J. Llopis, M. Whitney, G. Zlokarnik, R.Y. Tsien, Cell-permeant caged InsP3 ester shows that Ca2+ spike frequency can optimize gene expression, Nature, 392 (1998) 936-941.
[95] L. Lipskaia, M.L. Pourci, C. Delomenie, L. Combettes, D. Goudouneche, J.L. Paul, T. Capiod, A.M. Lompre, Phosphatidylinositol 3-kinase and calcium-activated transcription pathways are required for VLDL-induced smooth muscle cell proliferation, Circulation research, 92 (2003) 1115-1122.

[96] M. Yoeli-Lerner, G.K. Yiu, I. Rabinovitz, P. Erhardt, S. Jauliac, A. Toker, Akt blocks breast cancer cell motility and invasion through the transcription factor NFAT, Molecular cell, 20 (2005) 539-550.
[97] K.A. Bolanz, M.A. Hediger, C.P. Landowski, The role of TRPV6 in breast carcinogenesis, Mol Cancer Ther, 7 (2008) 271-279.
[98] E. Aydar, S. Yeo, M. Djamgoz, C. Palmer, Abnormal expression, localization and interaction of canonical transient receptor potential ion channels in human breast cancer cell lines and tissues: a potential target for breast cancer diagnosis and therapy, Cancer cell international, 9 (2009) 23.
[99] L. Tsavaler, M.H. Shapero, S. Morkowski, R. Laus, Trp-p8, a novel prostate-specific gene, is up-regulated in prostate cancer and other malignancies and shares high homology with transient receptor potential calcium channel proteins, Cancer research, 61 (2001) 3760-3769.
[100] H. Ouadid-Ahidouch, I. Dhennin-Duthille, M. Gautier, H. Sevestre, A. Ahidouch, TRP channels: diagnostic markers and therapeutic targets for breast cancer?, Trends in molecular medicine, (2012).
[101] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell, 144 (2011) 646-674.
[102] H. Fang, Y.A. Declerck, Targeting the tumor microenvironment: from understanding pathways to effective clinical trials, Cancer research, 73 (2013) 4965-4977.
[103] D. Hanahan, L.M. Coussens, Accessories to the crime: functions of cells recruited to the tumor microenvironment, Cancer cell, 21 (2012) 309-322.
[104] G. Lorusso, C. Ruegg, The tumor microenvironment and its contribution to tumor evolution toward metastasis, Histochemistry and cell biology, 130 (2008) 1091-1103.
[105] M. Moschetta, Y. Mishima, I. Sahin, S. Manier, S. Glavey, A. Vacca, A.M. Roccaro, I.M. Ghobrial, Role of endothelial progenitor cells in cancer progression, Biochim Biophys Acta, 1846 (2014) 26-39.
[106] S. Rafii, D. Lyden, R. Benezra, K. Hattori, B. Heissig, Vascular and haematopoietic stem cells: novel targets for anti-angiogenesis therapy?, Nature reviews. Cancer, 2 (2002) 826-835.
[107] J. Folkman, Role of angiogenesis in tumor growth and metastasis, Seminars in oncology, 29 (2002) 15-18.
[108] G.R. Mundy, Metastasis to bone: causes, consequences and therapeutic opportunities, Nature reviews. Cancer, 2 (2002) 584-593.
[109] J.H. Chamley, G.R. Campbell, J.D. McConnell, U. Groschel-Stewart, Comparison of vascular smooth muscle cells from adult human, monkey and rabbit in primary culture and in subculture, Cell and tissue research, 177 (1977) 503-522.
[110] J. Chamley-Campbell, G.R. Campbell, R. Ross, The smooth muscle cell in culture, Physiological reviews, 59 (1979) 1-61.
[111] J.H. Chamley-Campbell, G.R. Campbell, R. Ross, Phenotype-dependent response of cultured aortic smooth muscle to serum mitogens, The Journal of cell biology, 89 (1981) 379-383.
[112] J.H. Chamley-Campbell, G.R. Campbell, What controls smooth muscle phenotype?, Atherosclerosis, 40 (1981) 347-357.
[113] G.K. Owens, Regulation of differentiation of vascular smooth muscle cells, Physiological reviews, 75 (1995) 487-517.
[114] J. Thyberg, Differentiated properties and proliferation of arterial smooth muscle cells in culture, International review of cytology, 169 (1996) 183-265.
[115] S.M. Henshall, D.E. Afar, J. Hiller, L.G. Horvath, D.I. Quinn, K.K. Rasiah, K. Gish, D. Willhite, J.G. Kench, M. Gardiner-Garden, P.D. Stricker, H.I. Scher, J.J. Grygiel, D.B. Agus,

D.H. Mack, R.L. Sutherland, Survival analysis of genome-wide gene expression profiles of prostate cancers identifies new prognostic targets of disease relapse, Cancer research, 63 (2003) 4196-4203.
[116] L. Zhang, G.J. Barritt, Evidence that TRPM8 is an androgen-dependent Ca2+ channel required for the survival of prostate cancer cells, Cancer research, 64 (2004) 8365-8373.
[117] L. Zhang, G.J. Barritt, TRPM8 in prostate cancer cells: a potential diagnostic and prognostic marker with a secretory function?, Endocrine-related cancer, 13 (2006) 27-38.
[118] G. Bidaux, M. Roudbaraki, C. Merle, A. Crepin, P. Delcourt, C. Slomianny, S. Thebault, J.L. Bonnal, M. Benahmed, F. Cabon, B. Mauroy, N. Prevarskaya, Evidence for specific TRPM8 expression in human prostate secretory epithelial cells: functional androgen receptor requirement, Endocrine-related cancer, 12 (2005) 367-382.
[119] N. Bell, V. Hann, C.P. Redfern, T.R. Cheek, Store-operated Ca(2+) entry in proliferating and retinoic acid-differentiated N- and S-type neuroblastoma cells, Biochim Biophys Acta, 1833 (2013) 643-651.
[120] H.N. Jiang, B. Zeng, Y. Zhang, N. Daskoulidou, H. Fan, J.M. Qu, S.Z. Xu, Involvement of TRPC channels in lung cancer cell differentiation and the correlation analysis in human non-small cell lung cancer, PloS one, 8 (2013) e67637.
[121] K. Varga, K. Paszty, R. Padanyi, L. Hegedus, J.P. Brouland, B. Papp, A. Enyedi, Histone deacetylase inhibitor- and PMA-induced upregulation of PMCA4b enhances Ca2+ clearance from MCF-7 breast cancer cells, Cell calcium, 55 (2014) 78-92.
[122] A. Arbabian, J.P. Brouland, A. Apati, K. Paszty, L. Hegedus, A. Enyedi, C. Chomienne, B. Papp, Modulation of endoplasmic reticulum calcium pump expression during lung cancer cell differentiation, The FEBS journal, 280 (2013) 5408-5418.
[123] P. Ribiczey, A. Tordai, H. Andrikovics, A.G. Filoteo, J.T. Penniston, J. Enouf, A. Enyedi, B. Papp, T. Kovacs, Isoform-specific up-regulation of plasma membrane Ca2+ATPase expression during colon and gastric cancer cell differentiation, Cell calcium, 42 (2007) 590-605.
[124] P. Gelebart, T. Kovacs, J.P. Brouland, R. van Gorp, J. Grossmann, N. Rivard, Y. Panis, V. Martin, R. Bredoux, J. Enouf, B. Papp, Expression of endomembrane calcium pumps in colon and gastric cancer cells. Induction of SERCA3 expression during differentiation, The Journal of biological chemistry, 277 (2002) 26310-26320.
[125] T.H. Jang, S.H. Sun, Alterations in Ca2+ signaling, and c-fos and nur77 expression are associated with sodium butyrate-induced differentiation of C6 glioma cell, The Chinese journal of physiology, 43 (2000) 149-158.
[126] W.J. Lee, S.J. Roberts-Thomson, G.R. Monteith, Plasma membrane calcium-ATPase 2 and 4 in human breast cancer cell lines, Biochemical and biophysical research communications, 337 (2005) 779-783.
[127] Y.M. Usachev, S.L. Toutenhoofd, G.M. Goellner, E.E. Strehler, S.A. Thayer, Differentiation induces up-regulation of plasma membrane Ca(2+)-ATPase and concomitant increase in Ca(2+) efflux in human neuroblastoma cell line IMR-32, Journal of neurochemistry, 76 (2001) 1756-1765.
[128] J.P. Brouland, P. Gelebart, T. Kovacs, J. Enouf, J. Grossmann, B. Papp, The loss of sarco/endoplasmic reticulum calcium transport ATPase 3 expression is an early event during the multistep process of colon carcinogenesis, The American journal of pathology, 167 (2005) 233-242.
[129] B. Papp, J.P. Brouland, Altered Endoplasmic Reticulum Calcium Pump Expression during Breast Tumorigenesis, Breast cancer : basic and clinical research, 5 (2011) 163-174.
[130] X.Y. Xu, W.F. Gou, X. Yang, G.L. Wang, H. Takahashi, M. Yu, X.Y. Mao, Y. Takano, H.C. Zheng, Aberrant SERCA3 expression is closely linked to pathogenesis,

invasion, metastasis, and prognosis of gastric carcinomas, Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine, 33 (2012) 1845-1854.
[131] G. Christofori, New signals from the invasive front, Nature, 441 (2006) 444-450.
[132] P.S. Steeg, D. Theodorescu, Metastasis: a therapeutic target for cancer, Nature clinical practice. Oncology, 5 (2008) 206-219.
[133] F.M. Davis, T.A. Stewart, E.W. Thompson, G.R. Monteith, Targeting EMT in cancer: opportunities for pharmacological intervention, Trends in pharmacological sciences, (2014).
[134] L.C. Bailey-Downs, J.E. Thorpe, B.C. Disch, A. Bastian, P.J. Hauser, T. Farasyn, W.L. Berry, R.E. Hurst, M.A. Ihnat, Development and characterization of a preclinical model of breast cancer lung micrometastatic to macrometastatic progression, PloS one, 9 (2014) e98624.
[135] J. Sleeman, P.S. Steeg, Cancer metastasis as a therapeutic target, European Journal of Cancer, 46 (2010) 1177-1180.
[136] J. Lu, P.S. Steeg, J.E. Price, S. Krishnamurthy, S.A. Mani, J. Reuben, M. Cristofanilli, G. Dontu, L. Bidaut, V. Valero, G.N. Hortobagyi, D. Yu, Breast cancer metastasis: challenges and opportunities, Cancer research, 69 (2009) 4951-4953.
[137] I.J. Fidler, The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited, Nature reviews. Cancer, 3 (2003) 453-458.
[138] P.S. Steeg, Tumor metastasis: mechanistic insights and clinical challenges, Nature medicine, 12 (2006) 895-904.
[139] C. Coghlin, G.I. Murray, Current and emerging concepts in tumour metastasis, The Journal of pathology, 222 (2010) 1-15.
[140] K.W. Hunter, N.P. Crawford, J. Alsarraj, Mechanisms of metastasis, Breast cancer research : BCR, 10 Suppl 1 (2008) S2.
[141] C. Wei, X. Wang, M. Chen, K. Ouyang, L.S. Song, H. Cheng, Calcium flickers steer cell migration, Nature, 457 (2009) 901-905.
[142] J.P. Chen, Y. Luan, C.X. You, X.H. Chen, R.C. Luo, R. Li, TRPM7 regulates the migration of human nasopharyngeal carcinoma cell by mediating Ca(2+) influx, Cell calcium, 47 (2010) 425-432.
[143] P. Rybarczyk, M. Gautier, F. Hague, I. Dhennin-Duthille, D. Chatelain, J. Kerr-Conte, F. Pattou, J.M. Regimbeau, H. Sevestre, H. Ouadid-Ahidouch, Transient receptor potential melastatin-related 7 channel is overexpressed in human pancreatic ductal adenocarcinomas and regulates human pancreatic cancer cell migration, International journal of cancer. Journal international du cancer, 131 (2012) E851-861.
[144] 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.
[145] J.H. Kim, S. Lkhagvadorj, M.R. Lee, K.H. Hwang, H.C. Chung, J.H. Jung, S.K. Cha, M. Eom, Orai1 and STIM1 are critical for cell migration and proliferation of clear cell renal cell carcinoma, Biochemical and biophysical research communications, 448 (2014) 76-82.
[146] N. Yang, Y. Tang, F. Wang, H. Zhang, D. Xu, Y. Shen, S. Sun, G. Yang, Blockade of store-operated Ca(2+) entry inhibits hepatocarcinoma cell migration and invasion by regulating focal adhesion turnover, Cancer letters, 330 (2013) 163-169.
[147] L. Munaron, A. Fiorio Pla, Endothelial calcium machinery and angiogenesis: understanding physiology to interfere with pathology, Current medicinal chemistry, 16 (2009) 4691-4703.
[148] A.M. Patton, J. Kassis, H. Doong, E.C. Kohn, Calcium as a molecular target in angiogenesis, Current pharmaceutical design, 9 (2003) 543-551.
[149] A. Fiorio Pla, D. Avanzato, L. Munaron, I.S. Ambudkar, Ion channels and transporters in cancer. 6. Vascularizing the tumor: TRP channels as molecular targets, American journal of physiology. Cell physiology, 302 (2012) C9-15.

[150] R. Kalluri, R.A. Weinberg, The basics of epithelial-mesenchymal transition, The Journal of clinical investigation, 119 (2009) 1420-1428.
[151] J.P. Thiery, H. Acloque, R.Y. Huang, M.A. Nieto, Epithelial-mesenchymal transitions in development and disease, Cell, 139 (2009) 871-890.
[152] H. Hugo, M.L. Ackland, T. Blick, M.G. Lawrence, J.A. Clements, E.D. Williams, E.W. Thompson, Epithelial--mesenchymal and mesenchymal--epithelial transitions in carcinoma progression, Journal of cellular physiology, 213 (2007) 374-383.
[153] F.M. Davis, I. Azimi, R.A. Faville, A.A. Peters, K. Jalink, J.W. Putney, Jr., G.J. Goodhill, E.W. Thompson, S.J. Roberts-Thomson, G.R. Monteith, Induction of epithelial-mesenchymal transition (EMT) in breast cancer cells is calcium signal dependent, Oncogene, (2013).
[154] C. Gilles, M. Polette, J.M. Zahm, J.M. Tournier, L. Volders, J.M. Foidart, P. Birembaut, Vimentin contributes to human mammary epithelial cell migration, Journal of cell science, 112 (Pt 24) (1999) 4615-4625.
[155] K. Vuoriluoto, H. Haugen, S. Kiviluoto, J.P. Mpindi, J. Nevo, C. Gjerdrum, C. Tiron, J.B. Lorens, J. Ivaska, Vimentin regulates EMT induction by Slug and oncogenic H-Ras and migration by governing Axl expression in breast cancer, Oncogene, 30 (2011) 1436-1448.
[156] F.M. Davis, A.A. Peters, D.M. Grice, P.J. Cabot, M.O. Parat, S.J. Roberts-Thomson, G.R. Monteith, Non-stimulated, agonist-stimulated and store-operated Ca2+ influx in MDA-MB-468 breast cancer cells and the effect of EGF-induced EMT on calcium entry, PloS one, 7 (2012) e36923.
[157] F.M. Davis, P.A. Kenny, E.T. Soo, B.J. van Denderen, E.W. Thompson, P.J. Cabot, M.O. Parat, S.J. Roberts-Thomson, G.R. Monteith, Remodeling of purinergic receptor-mediated Ca2+ signaling as a consequence of EGF-induced epithelial-mesenchymal transition in breast cancer cells, PloS one, 6 (2011) e23464.
[158] 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, Biochemical and biophysical research communications, 411 (2011) 786-791.
[159] C.M. Perou, T. Sorlie, M.B. Eisen, M. van de Rijn, S.S. Jeffrey, C.A. Rees, J.R. Pollack, D.T. Ross, H. Johnsen, L.A. Akslen, O. Fluge, A. Pergamenschikov, C. Williams, S.X. Zhu, P.E. Lonning, A.L. Borresen-Dale, P.O. Brown, D. Botstein, Molecular portraits of human breast tumours, Nature, 406 (2000) 747-752.
[160] T. Sorlie, C.M. Perou, R. Tibshirani, T. Aas, S. Geisler, H. Johnsen, T. Hastie, M.B. Eisen, M. van de Rijn, S.S. Jeffrey, T. Thorsen, H. Quist, J.C. Matese, P.O. Brown, D. Botstein, P.E. Lonning, A.L. Borresen-Dale, Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications, Proceedings of the National Academy of Sciences of the United States of America, 98 (2001) 10869-10874.
[161] A. Prat, J.S. Parker, O. Karginova, C. Fan, C. Livasy, J.I. Herschkowitz, X. He, C.M. Perou, Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer, Breast cancer research : BCR, 12 (2010) R68.
[162] D.L. Holliday, V. Speirs, Choosing the right cell line for breast cancer research, Breast cancer research : BCR, 13 (2011) 215.
[163] R.J. Clifford, J.H. Kaplan, Human breast tumor cells are more resistant to cardiac glycoside toxicity than non-tumorigenic breast cells, PloS one, 8 (2013) e84306.
[164] R.M. Neve, K. Chin, J. Fridlyand, J. Yeh, F.L. Baehner, T. Fevr, L. Clark, N. Bayani, J.P. Coppe, F. Tong, T. Speed, P.T. Spellman, S. DeVries, A. Lapuk, N.J. Wang, W.L. Kuo, J.L. Stilwell, D. Pinkel, D.G. Albertson, F.M. Waldman, F. McCormick, R.B. Dickson, M.D. Johnson, M. Lippman, S. Ethier, A. Gazdar, J.W. Gray, A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes, Cancer cell, 10 (2006) 515-527.

[165] J. Greshock, K.E. Bachman, Y.Y. Degenhardt, J. Jing, Y.H. Wen, S. Eastman, E. McNeil, C. Moy, R. Wegrzyn, K. Auger, M.A. Hardwicke, R. Wooster, Molecular target class is predictive of in vitro response profile, Cancer research, 70 (2010) 3677-3686.
[166] G.R. Monteith, G.S. Bird, Techniques: high-throughput measurement of intracellular Ca(2+) -- back to basics, Trends in pharmacological sciences, 26 (2005) 218-223.
[167] W. Gobel, B.M. Kampa, F. Helmchen, Imaging cellular network dynamics in three dimensions using fast 3D laser scanning, Nature methods, 4 (2007) 73-79.
[168] D.A. Dombeck, M.S. Graziano, D.W. Tank, Functional clustering of neurons in motor cortex determined by cellular resolution imaging in awake behaving mice, The Journal of neuroscience : the official journal of the Society for Neuroscience, 29 (2009) 13751-13760.
[169] M.A. Busche, G. Eichhoff, H. Adelsberger, D. Abramowski, K.H. Wiederhold, C. Haass, M. Staufenbiel, A. Konnerth, O. Garaschuk, Clusters of hyperactive neurons near amyloid plaques in a mouse model of Alzheimer's disease, Science, 321 (2008) 1686-1689.
[170] R.Y. Tsien, A non-disruptive technique for loading calcium buffers and indicators into cells, Nature, 290 (1981) 527-528.
[171] A. Malgaroli, D. Milani, J. Meldolesi, T. Pozzan, Fura-2 measurement of cytosolic free Ca2+ in monolayers and suspensions of various types of animal cells, The Journal of cell biology, 105 (1987) 2145-2155.
[172] F. Di Virgilio, T.H. Steinberg, S.C. Silverstein, Inhibition of Fura-2 sequestration and secretion with organic anion transport blockers, Cell calcium, 11 (1990) 57-62.
[173] L. Homolya, Z. Hollo, U.A. Germann, I. Pastan, M.M. Gottesman, B. Sarkadi, Fluorescent cellular indicators are extruded by the multidrug resistance protein, The Journal of biological chemistry, 268 (1993) 21493-21496.
[174] K. Nooter, A.M. Westerman, M.J. Flens, G.J. Zaman, R.J. Scheper, K.E. van Wingerden, H. Burger, R. Oostrum, T. Boersma, P. Sonneveld, et al., Expression of the multidrug resistance-associated protein (MRP) gene in human cancers, Clinical cancer research : an official journal of the American Association for Cancer Research, 1 (1995) 1301-1310.
[175] V. Perez Koldenkova, T. Nagai, Genetically encoded Ca(2+) indicators: properties and evaluation, Biochim Biophys Acta, 1833 (2013) 1787-1797.
[176] A.E. Palmer, Y. Qin, J.G. Park, J.E. McCombs, Design and application of genetically encoded biosensors, Trends in biotechnology, 29 (2011) 144-152.
[177] J.E. McCombs, A.E. Palmer, Measuring calcium dynamics in living cells with genetically encodable calcium indicators, Methods, 46 (2008) 152-159.
[178] J.T. Russell, Imaging calcium signals in vivo: a powerful tool in physiology and pharmacology, British journal of pharmacology, 163 (2011) 1605-1625.
[179] L. Tian, S.A. Hires, T. Mao, D. Huber, M.E. Chiappe, S.H. Chalasani, L. Petreanu, J. Akerboom, S.A. McKinney, E.R. Schreiter, C.I. Bargmann, V. Jayaraman, K. Svoboda, L.L. Looger, Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators, Nature methods, 6 (2009) 875-881.
[180] Q. Chen, J. Cichon, W. Wang, L. Qiu, S.J. Lee, N.R. Campbell, N. Destefino, M.J. Goard, Z. Fu, R. Yasuda, L.L. Looger, B.R. Arenkiel, W.B. Gan, G. Feng, Imaging neural activity using Thy1-GCaMP transgenic mice, Neuron, 76 (2012) 297-308.
[181] S.D. Atkin, S. Patel, A. Kocharyan, L.A. Holtzclaw, S.H. Weerth, V. Schram, J. Pickel, J.T. Russell, Transgenic mice expressing a cameleon fluorescent Ca2+ indicator in astrocytes and Schwann cells allow study of glial cell Ca2+ signals in situ and in vivo, Journal of neuroscience methods, 181 (2009) 212-226.

Highlights

- Deregulated Ca^{2+} signaling is linked to each of the 'cancer hallmarks'.
- Altered Ca^{2+} transporter protein expression is associated with some cancers.
- Specific aspects of the Ca^{2+} signal appear to be altered in some cancer cells.
- The nature of altered Ca^{2+} signaling in cancer is not completely understood.
- Understanding altered Ca^{2+} signaling in cancer may guide therapeutic targeting.