



Review

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Author for correspondence:

Natalia Prevarskaya
e-mail: natacha.prevarskaya@univ-lille1.fr

Remodelling of Ca²⁺ transport in cancer: how it contributes to cancer hallmarks?

Natalia Prevarskaya¹, Halima Ouadid-Ahidouch², Roman Skryma¹
and Yaroslav Shuba^{3,4}

¹Inserm, U1003, Laboratoire de Physiologie Cellulaire, Equipe labellisée par la Ligue contre le cancer, Villeneuve d'Ascq, F-59650 France; Laboratory of Excellence, Ion Channels Science and Therapeutics; Université de Lille 1, Villeneuve d'Ascq, F-59650 France

²Laboratory of Cellular and Molecular Physiology, EA 4667: Ionic Channels in Breast Cancer, UFR of Sciences, UPJV, Amiens, France

³Bogomoletz Institute of Physiology and International Center of Molecular Physiology, NASU, Bogomoletz Street, 4, Kyiv-24 01024, Ukraine

⁴State Key Laboratory of Molecular and Cellular Biology, Kyiv, Ukraine

Cancer involves defects in the mechanisms underlying cell proliferation, death and migration. Calcium ions are central to these phenomena, serving as major signalling agents with spatial localization, magnitude and temporal characteristics of calcium signals ultimately determining cell's fate. Cellular Ca²⁺ signalling is determined by the concerted action of a molecular Ca²⁺-handling toolkit which includes: active energy-dependent Ca²⁺ transporters, Ca²⁺-permeable ion channels, Ca²⁺-binding and storage proteins, Ca²⁺-dependent effectors. In cancer, because of mutations, aberrant expression, regulation and/or subcellular targeting of Ca²⁺-handling/transport protein(s) normal relationships among extracellular, cytosolic, endoplasmic reticulum and mitochondrial Ca²⁺ concentrations or spatio-temporal patterns of Ca²⁺ signalling become distorted. This causes deregulation of Ca²⁺-dependent effectors that control signalling pathways determining cell's behaviour in a way to promote pathophysiological cancer hallmarks such as enhanced proliferation, survival and invasion. Despite the progress in our understanding of Ca²⁺ homeostasis remodelling in cancer cells as well as in identification of the key Ca²⁺-transport molecules promoting certain malignant phenotypes, there is still a lot of work to be done to transform fundamental findings and concepts into new Ca²⁺ transport-targeting tools for cancer diagnosis and treatment.

1. Introduction

Ca²⁺ signalling is imperative for normal cellular behaviour. In non-excitabile tissues, it is required for supporting specific cellular function, such as sensation, secretion, absorption, transcellular transport, as well as for maintaining stable tissue homeostasis characterized by balanced cell proliferation, cell death, cell motility, oxygen and nutrient supply. During carcinogenesis, Ca²⁺ signalling of some malignant cells is significantly remodelled in a way that compromises normal physiological functions at the same time enabling them to overwhelm normal cells by giving them unconditional advantages for uncontrolled multiplication, evasion of programmed cell death, adaptation to oxygen and nutrients sparse conditions, invasion and spreading beyond the primary tumour site. It would probably be an overstatement to assert that remodelling of Ca²⁺ signalling is the prime reason for such transformation in cell's behaviour; it is rather a consequence of dynamic changes in the genome, the influence of epigenetic, environmental factors or adaptive responses that provoke cancer. Nevertheless, as malignant remodelling of Ca²⁺ signalling helps to sustain cancer hallmarks, learning its intimate mechanisms and identifying the molecular players involved poses an opportunity for therapeutic halting the progression of certain hallmarks or even reversing them.

Cellular Ca^{2+} signalling takes a variety of forms in space and time. It is based on Ca^{2+} circulation among four primary compartments: extracellular space, cytoplasm, endoplasmic reticulum (ER, including Golgi) and mitochondrial, and involves numerous Ca^{2+} -handling and Ca^{2+} -sensing proteins specific for each compartment that provide for: (i) active energy-dependent Ca^{2+} transport between the compartments against a concentration gradient (Ca^{2+} pumps and coupled exchangers), (ii) passive Ca^{2+} streaming down a concentration gradient (Ca^{2+} -permeable ion channels), (iii) Ca^{2+} storage (Ca^{2+} -binding proteins), (iv) activation of downstream targets (Ca^{2+} -dependent effectors). Tightly controlled combined action of the proteins participating in Ca^{2+} -handling and formation of local Ca^{2+} -regulated signalling complexes enables Ca^{2+} signals coding in space and time for effective regulation of the processes as diverse as life and death [1].

Not only dynamic and spatially non-homogeneous changes of Ca^{2+} concentration in various compartments have signalling significance, but steady-state content of Ca^{2+} in the cytoplasm ($[\text{Ca}^{2+}]_C$), in the ER lumen ($[\text{Ca}^{2+}]_{ER}$), in mitochondrial matrix ($[\text{Ca}^{2+}]_M$) also impact a cell's fate. Indeed, sustained elevation of $[\text{Ca}^{2+}]_C$ in the form of overload, saturating all Ca^{2+} -dependent effectors, prolonged decrease in $[\text{Ca}^{2+}]_{ER}$, causing ER stress response, and high $[\text{Ca}^{2+}]_M$, inducing mitochondrial permeability transition (MPT), are considered to be pro-death factors [2–5]. At the same time such features of calcium homeostasis as (1) moderate to low basal $[\text{Ca}^{2+}]_C$ on which background cytosolic calcium oscillations, waves, sparks, spikes, flickers, etc. can occur, (2) substantial ER luminal Ca^{2+} content that warrants correct proteins' processing and uncompromised ability for Ca^{2+} release, and (3) low-level of $[\text{Ca}^{2+}]_M$ to sustain mitochondrial Ca^{2+} uptake for efficient maintenance of cell bioenergetics are thought to be important for supporting life-related processes such as secretion, proliferation, differentiation and motility [6–11]. Even the a steady-state extracellular calcium ($[\text{Ca}^{2+}]_O$) level has significant value role for normal tissue homeostasis and prevention of tumourigenesis [12].

In cancer, because of mutations, aberrant expression, regulation and/or subcellular targeting of Ca^{2+} -handling/transport protein(s), Ca^{2+} signalling can become distorted. This causes deregulation of Ca^{2+} -dependent effectors promoting certain pathophysiological cancer hallmark(s). Most of the relevant data were obtained in the studies on primary cancer cells and cancer cell lines derived from common solid tumours. However, as the mechanisms of Ca^{2+} homeostasis and signalling in haematocytes are rather similar to other non-excitatory cells, the general conclusions regarding pro-malignant remodelling of Ca^{2+} signalling are probably applicable to the haematological malignancies as well.

It should be noted, however, that the role of Ca^{2+} -transport proteins in cancer is context-specific, and the same transporter, depending on the presence of certain regulatory signals and/or partner proteins, and formation of local signalling complexes may participate in the promotion of different cancer hallmarks and even several simultaneously [13].

2. Ca^{2+} remodelling that promotes proliferation

Quiescent cells are commonly characterized by very localized, modest in size and short-lived in time $[\text{Ca}^{2+}]_C$ increases taking the form of sparks, spikes, puffs, flickers [6–11], which are necessary to sustain baseline physiological activity. Switching

from a quiescent to an active proliferative state in response to mitogenic growth signals involves global dynamic $[\text{Ca}^{2+}]_C$ elevations owing to activation and complex interactions of all molecular components of the Ca^{2+} -handling toolkit specific for a given cell type. Among the direct effectors for such Ca^{2+} signalling are calmodulin (CaM) and Ca^{2+} /calmodulin-dependent protein kinases II (CaMKII), protein phosphatase 2B (calcineurin) and protein kinase C (PKC), which in turn regulate activation of transcription factors controlling the cascade of genes expression required for cell-cycle progression [14].

In cancer, it is not only the proliferation of tumour cells that stops depending solely on external growth signals via development of significant growth autonomy [15,16], but the Ca^{2+} -handling toolkit undergoes profound remodelling (figure 1) to favour activation of Ca^{2+} -dependent transcription factors, such as the nuclear factor of activated T cells (NFAT), c-Myc, c-Jun, c-Fos that promote hypertrophic growth via induction of the expression of the G1 and G1/S phase transition cyclins (D and E) and associated cyclin-dependent kinases (CDK4 and CDK2) [14]. Consistent with this notion, it was shown that the proliferation-promoting action of $\alpha 1$ -adrenoreceptor ($\alpha 1$ -AR) agonists on prostate cancer (PCa) cells is based on preferred coupling of the $\alpha 1$ -AR-coupled signalling pathway to activation of a member of transient receptor potential (TRP) channel family, TRPC6, Ca^{2+} influx through which in turn specifically regulates NFAT in a Ca^{2+} /calcineurin-dependent manner [17]. In addition, in LNCaP PCa cells yet another highly oncogenic TRP member, TRPV6 [18], was shown to support high proliferation rates apparently by providing constitutive Ca^{2+} influx required for subsequent downstream NFAT activation [19]. TRPV6 expression in PCa cells is also positively regulated by vitamin D3 receptor (VDR) activation to enhance $[\text{Ca}^{2+}]_C$ and proliferation of cells [20].

In MCF-7 breast cancer cells, TRPV6 function required for supporting both proper $[\text{Ca}^{2+}]_C$ and cell proliferation was shown to depend on interaction with its newly identified partner protein, Numb1, which negatively regulates TRPV6 activity [21], highlighting the significance of channel regulators in determining their oncogenic potential.

Constitutive activation of the Ca^{2+} /calcineurin/NFAT signalling pathway has been also implicated in the mechanism of upregulated transcription of oncogenic *c-myc* in pancreatic carcinomas, ultimately resulting in accelerated G1/S phase transition, increased cell proliferation and enhanced anchorage-independent growth [22], although the mechanisms for elevated $[\text{Ca}^{2+}]_C$ in these cells remain undetermined. In MCF-7 breast cancer cells, G1 phase progression and G1/S transition were shown to depend on the ORAI3 Ca^{2+} -permeable channel that contributes to store-operated Ca^{2+} entry (SOCE) in these cells [23,24]. It positively regulates the expression of cyclins (D1, E), CDK4 and 2, and suppresses cyclin-dependent kinase inhibitors (CDKIs) such, p21 and p53 by regulating the expression and the activity of *c-myc* [24,25].

Many Ca^{2+} -transport proteins have been implicated in the proliferation of cancer cells, including sarco(endo)plasmic reticulum (SERCA) [26], the Golgi network secretory pathway (SPCA) [27] and plasma membrane (PMCA) [28] Ca^{2+} -ATPases (pumps), the inositol 1,4,5-trisphosphate receptor (IP₃R) [29,30] and ryanodine receptor (RyR) [31] Ca^{2+} release channels of the ER, STIM and ORAI constituents of plasmalemmal store-operated (SOC) channels [13,23–25,32,33], T-type voltage-gated calcium channels (VGCCs) [34,35],

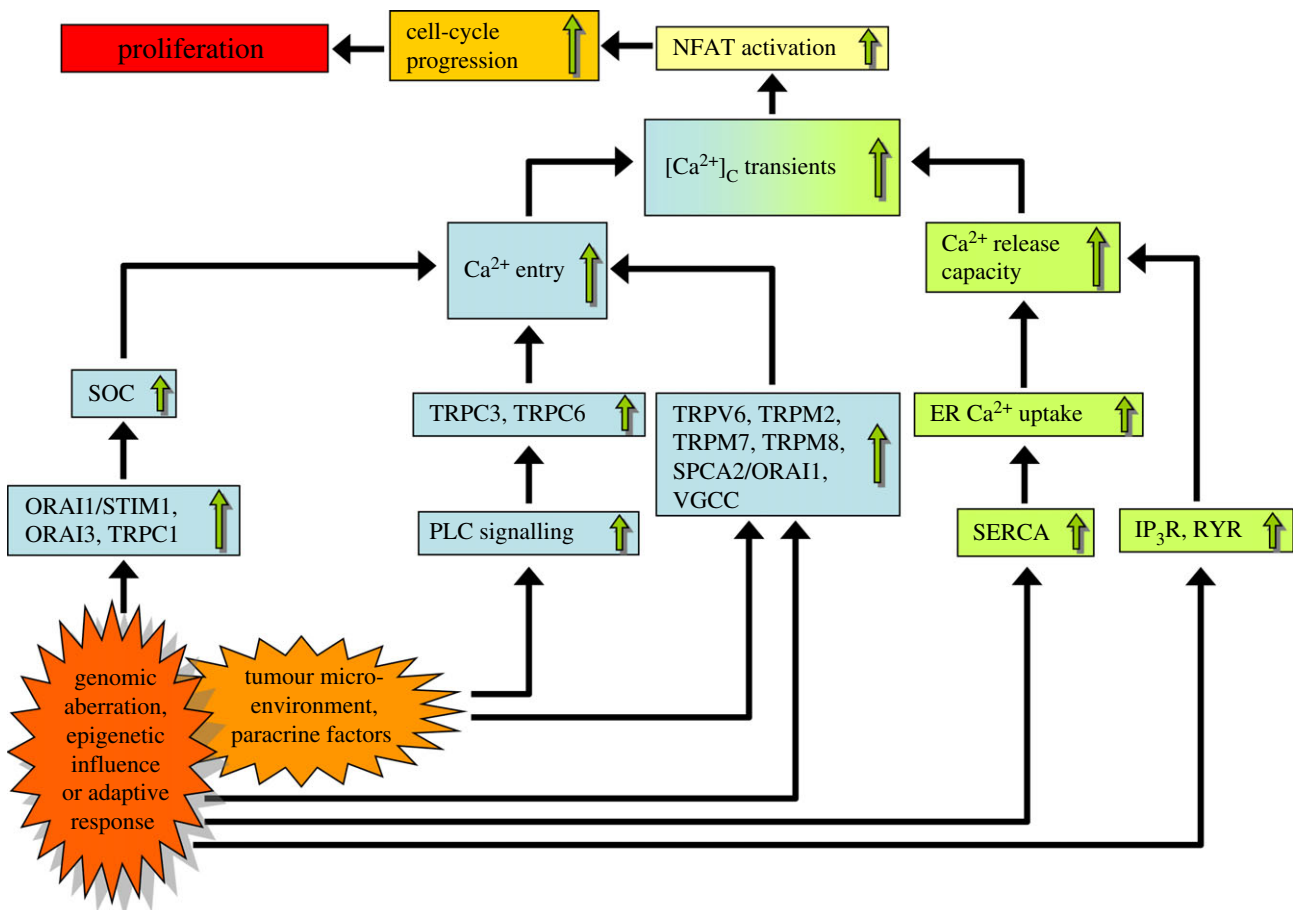


Figure 1. Ca^{2+} transport remodelling in promotion of cancer cell proliferation. Black arrows show the sequence of events; step-by-step following of black arrows enables a specific pathway to be traced; upward arrows in the boxes indicate increased expression and/or function of certain protein(s) or stimulation of a specified process. The boxes related to Ca^{2+} entry and Ca^{2+} release mechanisms are coloured in blue and green, respectively. See text for details. ER, endoplasmic reticulum; IP_3R , inositol triphosphate receptor-channel; NFAT, nuclear factor of activated T cells; ORAI1 and ORAI3, channel-forming proteins of SOC; PLC, phospholipase C; SERCA, sarco(endo)plasmic reticulum calcium ATPase; SOC, store-operated channels; SPCA2, secretory pathway calcium ATPase 2; STIM1, stromal interaction molecule 1 (ER Ca^{2+} sensor in SOC activation); TRP, various members of transient receptor potential channel family; RYR, ryanodine receptor-channel; VGCC, voltage-gated calcium channels. (Online version in colour.)

various TRP-members [36] such as TRPV6 [19], TRPC1, TRPC3 and TRPC6 [17,37–40], TRPM2 [41], TRPM7 [40,42], TRPM8 [40,43] (figure 1). Enhanced proliferation of cancer cells is commonly correlated with higher expression of those proteins from the Ca^{2+} -handling toolkit which participate in $[\text{Ca}^{2+}]_C$ increases by providing Ca^{2+} influx or Ca^{2+} release, or which sustain ER Ca^{2+} filing.

3. Ca^{2+} remodelling in conferring apoptosis resistance

The Ca^{2+} -dependence of apoptosis is well defined in numerous original studies and comprehensively illuminated in numerous review articles [2–5]. It commonly involves initial cytosolic Ca^{2+} overload owing to massive entry and/or vast release and can subsequently progress via three largely inter-related and interdependent pathways: mitochondrial, cytoplasmic and ER stress-related (reviewed in [2–5,44]). Thus, cancer cells may evade apoptosis through decreasing calcium influx into the cytoplasm. This can be achieved by either downregulation of the expression of plasma membrane Ca^{2+} -permeable ion channels or by reducing the effectiveness of the signalling pathways that activate these channels. Such protective measures would largely diminish the possibility of Ca^{2+} overload in response to pro-apoptotic stimuli, thereby

impairing the effectiveness of mitochondrial and cytoplasmic apoptotic pathways. Yet another defence mechanism against apoptosis would involve cancer cell adaptation to the reduced basal $[\text{Ca}^{2+}]_{ER}$ without induction of pro-apoptotic ER stress response that usually accompanies ER luminal calcium imbalance.

In full agreement with these general considerations, it was shown that PCa cells, upon transition to more aggressive androgen-independent phenotype, which is characterized by substantial enhancement of cell survival, downregulate their SOCE by decreasing the expression of the principal plasma membrane SOC-channel-forming subunit, ORAI1 protein [45], as well as of the ER Ca^{2+} sensor regulating SOC activation, STIM1 protein [46] (figure 2). Moreover, as the ER luminal Ca^{2+} -binding protein calreticulin presents androgen-response gene in the prostate [47], its lowered expression in androgen-independent PCa cells compromises the Ca^{2+} storage capacity of the ER and initiates a chain of adaptive responses in the expression of other ER Ca^{2+} -handling proteins to keep ER Ca^{2+} filling at a lower level [48,49]. The latter include lowered SERCA2b expression to reduce Ca^{2+} uptake and higher expression of ER-resident Bcl-2 protein that is likely to promote Ca^{2+} leak from the ER [48] (figure 2).

However, the Ca^{2+} -handling toolkit in apoptosis resistant cancer cell phenotypes undergoes remodelling not only in a way that limits Ca^{2+} influx and maintains low $[\text{Ca}^{2+}]_{ER}$. There

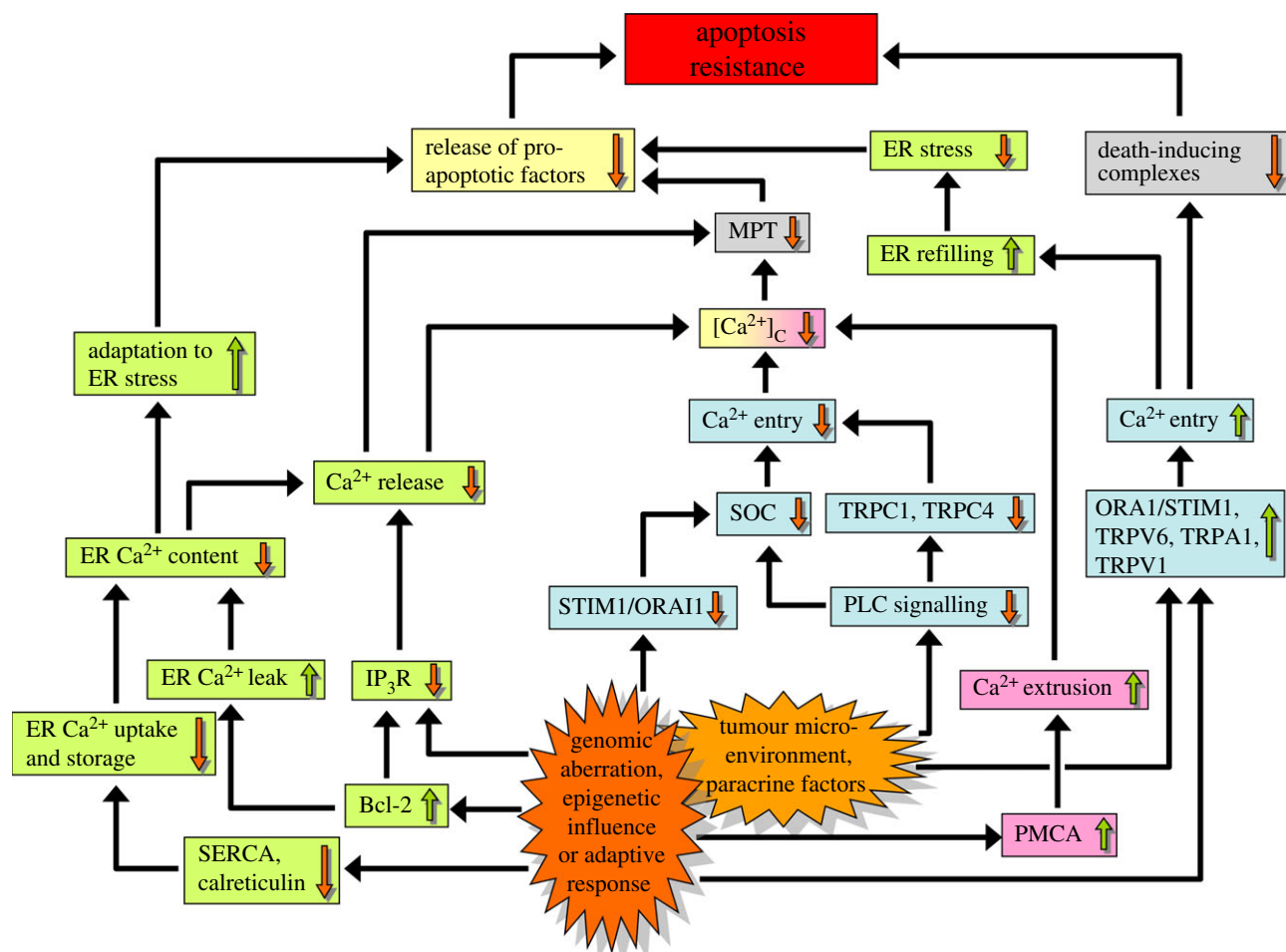


Figure 2. Ca^{2+} transport remodelling in conferring apoptosis resistance in cancer cells. Black arrows show the sequence of events; step-by-step following of black arrows enables a specific pathway to be traced; green upward arrows in the boxes indicate increased expression and/or function of certain protein(s) or stimulation of a specified process; orange downward arrows in the boxes indicate decreased expression and/or function of certain protein(s) or inhibition of a specified process. The boxes related to Ca^{2+} entry, Ca^{2+} release and Ca^{2+} extrusion mechanisms are coloured in blue, green and magenta, respectively. See text for details. Abbreviations in addition to those specified for figure 1. MPT, mitochondrial permeability transition; PMCA, plasma membrane calcium ATPase. (Online version in colour.)

are number of instances when apoptosis resistance is conferred by higher expression of Ca^{2+} entry channels (figure 2). Indeed, it was shown that TRPV6 and ORAI3 not only promote proliferation, but also increase survival of PCa and breast cancer cells, respectively [19,20,24]. ORAI3-conferred survival of breast cancer cells involved Ca^{2+} -dependent increase of c-Myc expression and activity resulting in the inhibition of pro-apoptotic Bax protein expression [24,25]. It was also demonstrated that higher levels of TRPA1 channel confer apoptosis resistance and promote survival of small cell lung carcinoma (SCLC) cells via TRPA1-mediated Ca^{2+} entry leading to stimulation of ERK1/2 via Src [50].

Enhanced apoptosis resistance of cancer cells associated with overexpression of certain types of plasma membrane Ca^{2+} -permeable channels may in part result from the formation of localized ' Ca^{2+} -dependent anti-apoptotic signalling complexes' to which these channels provide a preferred, spatially restricted supply of Ca^{2+} . An example of such relationships was described in leukaemic T cells, in which SOC-channel-forming ORAI1 protein and ORAI1-activating ER Ca^{2+} sensor STIM1 co-localize with CD95/FADD/caspase death-inducing signalling complex (DISC) in the confined plasma membrane microdomains to which ORAI1 provides polarized Ca^{2+} entry [51] (figure 2).

Yet another mechanism for the enhanced apoptosis resistance of cancer cells due to overexpression of plasma membrane Ca^{2+} -permeable channels may in part involve the

reduction of the significance of Ca^{2+} -dependent ER-stress response apoptosis initiation, as Ca^{2+} entry through the channel may serve as a source of Ca^{2+} to sustain ER store refilling (figure 2). Consistent with this notion, expression of the TRPV1 channel is positively correlated with grading of human glioma (astrocytoma) [52,53]. However, exogenous TRPV1 agonists, as well as its endogenous agonists, endocannabinoids, were shown to trigger the apoptosis of glioma cells, not apparently because of Ca^{2+} entry via plasma membrane-localized TRPV1, but primarily via ER-stress owing to TRPV1 localization in the ER membrane [52].

Mitochondrial Ca^{2+} uptake evoked by pronounced Ca^{2+} entry or IP₃R-mediated surges of $[\text{Ca}^{2+}]_c$ can trigger MPT and, in turn, the release of mitochondrial apoptosis-inducing factors. MPT, which is specifically associated with IP₃R activation, relies on privileged Ca^{2+} signal transmission from IP₃R to mitochondria in the ER-mitochondrial contact sites. Thus, the enhanced apoptosis resistance of cancer cells must involve downregulated expression or activation IP₃R that compromise IP₃R-mediated release (figure 2). Consistent with this notion, in bladder cancer cells the acquisition of cisplatin resistance was shown to result from cisplatin-induced downregulation of IP₃R1 expression [54]. Moreover, the common anti-apoptotic protein Bcl-2 can directly interact with IP₃R and inhibit channel opening and ER Ca^{2+} release, thereby contributing to the reduction of Ca^{2+} -mediated apoptosis [55,56] (figure 2).

An anti-apoptotic role has also been ascribed to the expression of PMCA2 and PMCA4 calcium pumps in breast cancer cells [57,58,59]. Interestingly, PMCA2-induced apoptosis resistance is by direct interaction with calcineurin leading to NFAT inhibition and concomitant decrease in NFAT-dependent expression of pro-apoptotic Fas ligand (FasL) [58], while PMCA4 regulates the NF κ B nuclear translocation [59].

Cancer cell survival can be also enhanced by autophagy, an intracellular self-digestive process that normally maintains the balance between the synthesis, degradation and subsequent recycling of cellular products. In advanced cancer, it participates in selection of the cells with increased tolerance to tumour-specific hypoxia and nutrient deficiency subsequent to disorganized angiogenesis. As a result, metastatic potential and resistance to anti-cancer therapy will probably increase.

The IP₃R calcium release channel of the ER is considered a key player in autophagy [60,61]. The IP₃R is known to reside in the ER membranes within ER-mitochondria contact sites and to provide basal constitutive low-level Ca²⁺ signalling to sustain mitochondrial Ca²⁺ uptake required for efficient mitochondrial respiration and maintenance of normal cell bioenergetics [61]. Blocking IP₃Rs or suppressing IP₃ production abolishes this signal, leading to lowered ATP production and an increased AMP/ATP ratio which in turn activates AMPK and induces autophagy [62]. Thus, disruption of IP₃R-mediated ER-mitochondria crosstalk and compromised cell bioenergetics which may take place in advanced cancer under hypoxic and nutrient-deficient conditions may activate autophagy as pro-survival mechanism.

4. Ca²⁺ remodelling in promotion cell migration and metastasis

Metastatic dissemination from the primary tumour site to multiple tissues is the main cause of mortality in cancer. Tumour cells become invasive by acquiring high migratory potential along with the increased ability to degrade extracellular matrix (ECM).

Ca²⁺ signalling is critical for regulating cell migration and invasion. In malignant cells, it is remodelled in a way that promotes the turnover of focal adhesions, enhances contractile forces and facilitates proteolysis of ECM components [63]. Ca²⁺-dependent regulation of the molecular machinery of migration is provided by calcineurin, CaMKII, proline-rich tyrosine kinase-2 (PYK2), Ca²⁺-dependent protease, calpain, which regulate focal adhesion dynamics and S100 family of EF-hand calcium-binding proteins (especially S100A4) which promotes cell migration via interaction with cytoskeletal proteins, including actin [63].

The links among the expression and function of certain Ca²⁺ permeable channels and cancer cells migration, invasion and metastasis are still largely phenomenological, and the mechanisms of their involvement are not fully understood. The following Ca²⁺ permeable channels have been implicated in the enhanced migration of various types of cancer cells (figure 3): TRPC1 [64], TRPM7 [65–71], TRPM8 [43,72,73], TRPV1 [74], TRPV2 [75], TRPV6 [40], STIM1 and ORAI1 SOC constituents [13,76–79], some types of VGCCs [35,80]. Owing to the presence of mechanical stimulus-dependent and Mg-ATP-dependent modes of activation TRPM7 was especially implicated in providing spatially restricted Ca²⁺ entry in response to local membrane stretch

in front of migrating cells [66,69] as well as promoting m-calpain-mediated disassembly of peripheral adhesions under decreased intracellular Mg-ATP levels [65] typically found in hypoxic tumour conditions. Moreover, high levels of TRPM7 expression *per se* was established as a prognostic marker for breast cancer [70] and pancreatic ductal adenocarcinoma (PDAC) [71] progression. The presence of TRPM7 was shown to be directly linked to metastasis formation in a mouse xenograft model of human breast cancer [70] and PDAC cell migration [71]. In MDA-MB-231 breast cancer cells, TRPM7 was found to regulate myosin II-based cellular tension, thereby modifying focal adhesion number, cell-cell adhesion and polarized cell movement [70].

High pro-migratory and pro-invasion potentials have been also ascribed to TRPV1, TRPV2, TRPV6 and TRPM8 channels (figure 3). Higher expression of TRPV2 in PCa cells or TRPM8 in squamous cell carcinoma correlated with the induction of MMP-2, MMP-9 and cathepsin B [73,75]. Using laser capture microdissection of breast tumour tissue, it was established that higher expression of TRPV6 channel tend to localize in the invasive areas, compared with the non-invasive ones, and TRPV6 silencing was able to inhibit migration and invasion of breast cancer cells [40]. However, with respect to TRPV1 channel diverging results have been reported, suggesting that its role in malignant motility and invasion may be cancer cell-specific. Indeed, activation of the TRPV1 channel was shown to promote migration of human hepatoblastoma cells in response to hepatocyte growth factor treatment [74]. On the other hand, in urothelial cancer TRPV1 downregulation rather than enhancement was found to correlate with more aggressive and invasive tumour phenotype suggesting that such downregulation may present an independent negative prognostic factor for bladder cancer patients [81].

Recently, SOCE and its STIM and ORAI constituents have emerged as important regulators of malignant cell migration (figure 3). Enhanced STIM1-ORAI1-mediated SOCE promoted higher rate of focal adhesion turnover and fast migration of metastatic breast cancer cells via activation of GTPases Ras and Rac [76] and of cervical cancer cells via engagement of calpain and PYK2 [13]. Higher migration of cervical cancer cells owing to STIM1 overexpression was shown to correlate with its accumulation in the ER punctae translocated towards plasma membrane of migratory cells and increasing cytosolic Ca²⁺ spikes [78]. This resulted in more effective recruitment and association of active focal adhesion kinase (pTyr397-FAK) and talin at focal adhesions to facilitate force transduction from integrin signalling, and to promote actomyosin formation [78].

Ca²⁺-entry pathways often interact with Ca²⁺ release mechanisms to produce [Ca²⁺]_C signals required for migration. For instance, localized [Ca²⁺]_C flickers at the front of migrating fibroblasts occurred because of interaction of TRPM7-mediated Ca²⁺ influx and Ca²⁺ release via type 2 IP₃R (IP₃R2) [66,69], whereas global [Ca²⁺]_C increase associated with enhanced migration of nasopharyngeal carcinoma cells depended on activation of TRPM7 and CICR involving RyRs [67] (figure 3).

Of other Ca²⁺ release channels, type 3 IP₃R (IP₃R3) was implicated in the invasive behaviours of glioblastoma cells [82] and in the peritoneal dissemination of gastric cancers [29]. IP₃R3 was shown to be over-expressed in glioblastoma cells, and its pharmacological or siRNA-mediated inhibition suppressed [Ca²⁺]_C increases and migration of glioblastoma cells with IP₃R3 inhibition by caffeine representing the most effective pharmacological tool [82].

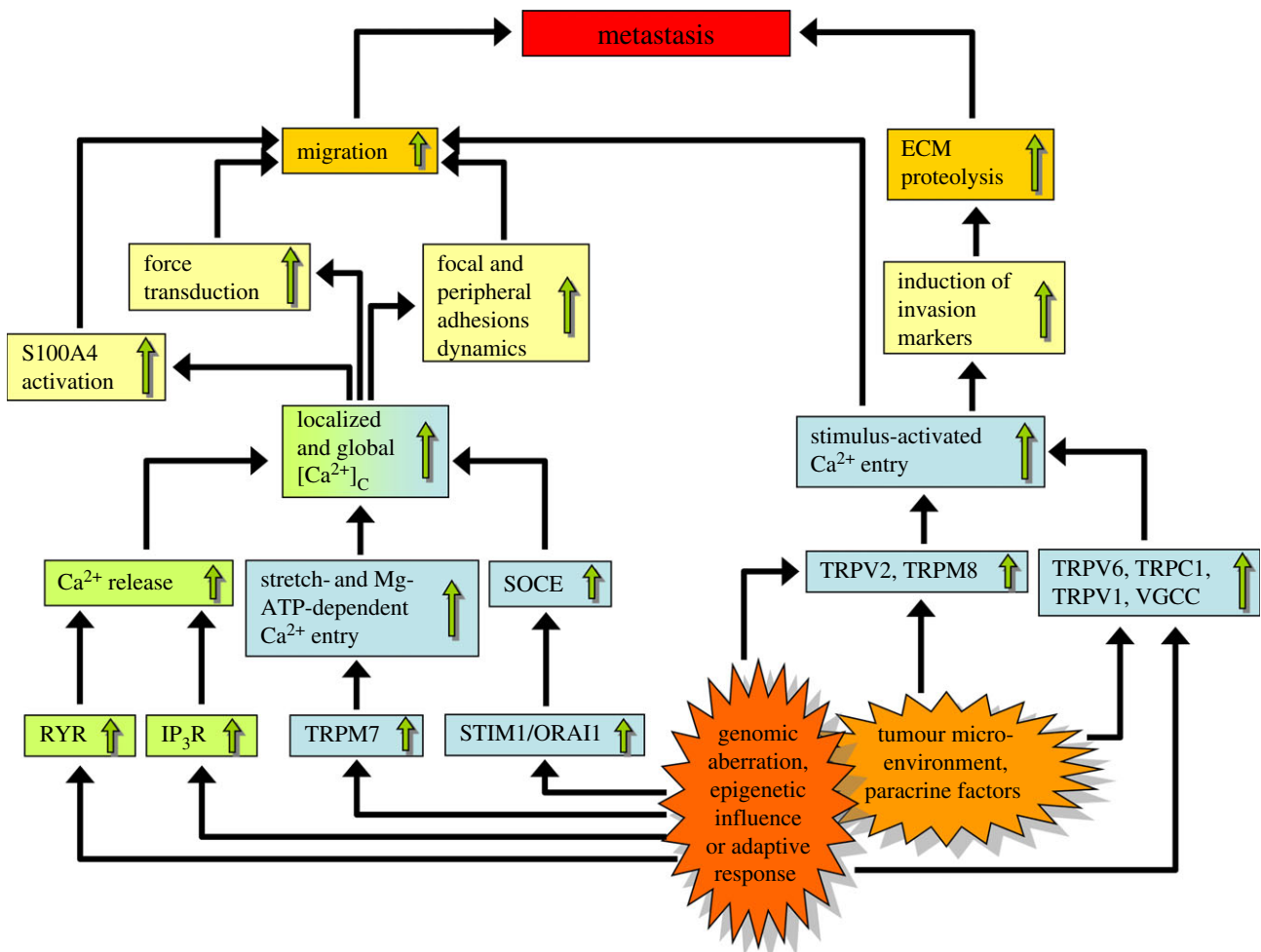


Figure 3. Ca^{2+} transport remodelling in the promotion of cancer cell metastasis. Black arrows show the sequence of events; step-by-step following of black arrows enables a specific pathway to be traced; green upward arrows in the boxes indicate increased expression and/or function of certain protein(s) or stimulation of a specified process. The boxes related to Ca^{2+} entry and Ca^{2+} release mechanisms are coloured in blue and green, respectively. See text for details. Abbreviations in addition to those specified for figures 1 and 2: ECM, extracellular matrix; SOCE, store-operated calcium entry. (Online version in colour.)

In addition, $[\text{Ca}^{2+}]_{\text{C}}$ increase owing to mobilization from ER stores was shown to promote migration of HeLa and MDA-MB-231 cancer cells via Ca^{2+} -dependent activation of S100A4 (figure 3) and facilitation of its interaction with target cytoskeletal proteins [83].

5. Ca^{2+} remodelling in tumour vascularization

Vascularization is critical for tumour growth and metastasis. Angiogenesis relies on proliferation and motility of vascular endothelial cells (ECs), which can be activated by diverse extracellular signals. Tumour cells secrete several peptides and growth factors with mitogenic or pro-angiogenic effects on ECs *in vitro* and *in vivo*, such as endothelin-1, angiotensin II, basic fibroblast growth factor (bFGF) as well as the vascular endothelial growth factor (VEGF) family of proteins, which are the most potent EC mitogens [84–86]. These factors can act in a paracrine-type manner owing to their release by tumour and stroma cells as well as in an autocrine fashion on ECs.

Intracellular Ca^{2+} signals are involved at different critical phases of regulation of the complex process of angiogenesis and tumour progression [87–89], and VEGF acts directly on ECs to induce Ca^{2+} signalling involving Ca^{2+} entry and IP_3R -mediated release [90–92]. Carboxyamidotriazole (CAI), an orally active agent with anti-neoplastic and anti-angiogenic activity, is one of the few anti-cancer agents with clear Ca^{2+}

homeostasis-targeting properties. It is known to act via disruption of Ca^{2+} -mediated signal transduction owing to inhibition of non-voltage-operated Ca^{2+} channels, which causes suppression of VEGF signalling in ECs, endothelial proliferation and angiogenesis [90]. CAI inhibits $[\text{Ca}^{2+}]_{\text{C}}$ increases during VEGF-A-induced EC proliferation [90], consistent with the requirement of Ca^{2+} influx for angiogenesis. This agent demonstrated the potential to inhibit tumour cell growth, invasion and metastasis, and clinical trials [93] suggest that it may have utility as a maintenance therapeutic agent for some types of cancer.

CAI appeared to be quite specific for SOC channels [94], which in ECs are composed of STIM1 and ORAI1 proteins [95]. Thus, abnormalities in the expression and/or function of STIM1 and ORAI1, which were shown to mediate VEGF-evoked calcium entry promoting EC migration, tube formation and angiogenesis [91], and might be involved in the enhancing the pro-angiogenic response of ECs in tumours (figure 4). Indeed, upregulated mRNA and protein levels of ORAI1, STIM1 and TRPC1 (which is also known to contribute to SOCE) accompanied by the increase in CAI-sensitive SOCE were detected in endothelial progenitor cells from patients with renal cellular carcinoma [96].

A number of the TRP-channel family members are expressed in ECs contributing the angiogenic process by providing agonist- or mechanical stretch-induced Ca^{2+} entry [85,88,97] (figure 4). In particular, TRPC1 and TRPC4 channels, which exhibit a store-dependent mode of gating, have been implicated

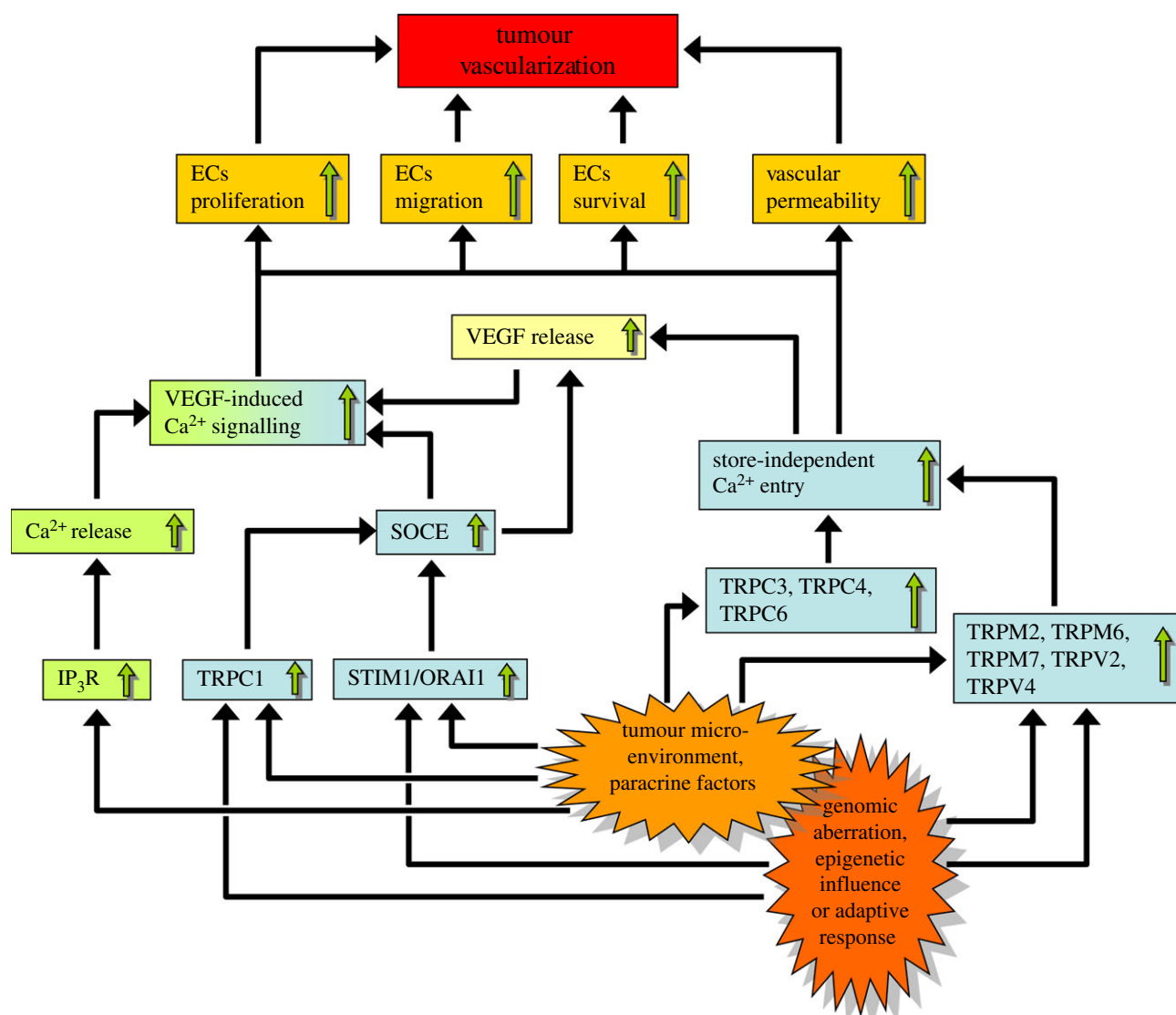


Figure 4. Ca^{2+} transport remodelling in tumour vascularization. Black arrows show the sequence of events; step-by-step following of black arrows enables a specific pathway to be traced; green upward arrows in the boxes indicate increased expression and/or function of certain protein(s) or stimulation of a specified process. The boxes related to Ca^{2+} entry and Ca^{2+} release mechanisms are coloured in blue and green, respectively. See text for details. Abbreviations in addition to those specified for figures 1–3: ECs, vascular endothelial cells; VEGF, vascular endothelial growth factor. (Online version in colour.)

in Ca^{2+} influx required for the pro-angiogenic response of ECs. The involvement of diacylglycerol-gated TRPC3 or TRPC6 was implicated in VEGF-induced permeability of microvascular ECs [98,99]. ECs also express members of TRP family (TRPM and TRPV), as well as STIM and ORAI involved in different aspects of malignant pro-angiogenic behaviours such as EC proliferation, migration and permeability [88,89,97] (figure 4). In addition to directly promoting EC proliferation, migration and permeability, Ca^{2+} influx through TRPs might stimulate ECs to produce and release the angiogenic growth factors VEGF, FGF and PDGF, which in turn might stimulate angiogenesis in an autocrine or paracrine manner (figure 4). It was also shown that Ca^{2+} influx through the reverse mode of the NCX1 $\text{Na}^+/\text{Ca}^{2+}$ exchanger is required for VEGF-induced ERK1/2 phosphorylation and downstream EC functions in angiogenesis [100].

6. Clinical prospects

Despite significant achievements in understanding remodelling of Ca^{2+} signalling in cancer progression and cancer

hallmarks, the practical utility of these findings for clinicians still remains very limited [101]. Given the ubiquity of the Ca^{2+} handling toolkit and its involvement in the physiological activity of normal cells, selective targeting of its components in cancer cells presents the major challenge. Despite the existence of numerous pharmacological and molecular biological research tools for targeting Ca^{2+} transport proteins with the purpose of reducing or reversing malignant behaviours of cancer cells in *in vitro* conditions with some of them even demonstrating profound anti-neoplastic effects in *in vivo* animal models, because of poor specificity, significant adverse effects and/or problems with selective delivery these research tools cannot be used clinically.

In addition to the prospective clinical tools and strategies described before [101], recently synthetic vanilloids were proposed as therapeutics for high-grade astrocytoma [52]. By activating the ER-localized TRPV1 channel, which is significantly over-expressed in this type of cancer cell, they induce ER-stress thereby promoting tumour cell death [52]. As prolonged cytosolic Ca^{2+} overload and/or ER Ca^{2+} depletion represent strong pro-death factors, overactivating the existing Ca^{2+} -permeable channels in cancer cells seems in general

to be a promising approach for cancer treatment. One of the realizations of this approach is based on using SERCA pump inhibitors from plant-derived sesquiterpene lactones and their derivatives which promote Ca^{2+} -dependent apoptosis by preventing ER Ca^{2+} uptake [102]. As systemic administration of these compounds can produce significant toxicity to normal cells, to enable their use for PCa treatment it was proposed that they should be combined with a targeting peptide representing the substrate for prostate cancer-specific serine protease, prostate-specific antigen [103].

7. Conclusion

Remodelling of Ca^{2+} signalling in cancer helps to sustain most of the cancer hallmarks. Identification of key Ca^{2+} -transport molecules which altered expression and/or function underlies pathological changes provides promising targets for cancer treatment. However, the ubiquity of molecular players

involved in maintaining normal as well as pathologic Ca^{2+} homeostasis so far prevents their selective targeting in cancer cells without significant adverse effects. Although Ca^{2+} transport is still a novel area of research in oncology, rapid development of the field ensures that improved molecular Ca^{2+} transport-targeting tools for cancer diagnosis and treatment will eventually be developed.

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