Alterations of calcium homeostasis in cancer cells
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Typical hallmarks of cancer include programmed cell death evasion, uncontrolled cell growth, invasion, and metastasis. Changes in intracellular Ca²⁺ levels can modulate signaling pathways that control a broad range of cellular events, including those important to tumorigenesis and cancer progression. Here we discuss how known molecular mediators of cellular Ca²⁺ homeostasis impact tumor dynamics and how deregulation of major oncogenes and tumor suppressors is tightly associated with Ca²⁺ signaling.

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Introduction
In 1944, Carruthers and Sunzteff described for the first time a direct correlation between Ca²⁺ and cancer, showing that a reduction in Ca²⁺ levels in hyperplastic mouse epidermis was an important feature in precancerous conditions [1]. More than 70 years later, the study of Ca²⁺ dynamics in both carcinogenesis and tumor progression is considered a key aspect of cancer biology. Spectacular advances in the understanding of intracellular Ca²⁺ signaling pathways have been made in recent years, leading to the identification of important molecular players that are now the subjects of thorough mechanistic investigations. The remodeling of intracellular Ca²⁺ homeostasis, as a cause or consequence of the activity of different cancer-related proteins with altered functions, is now thought of as a general hallmark of cancer cells.

Intracellular Ca²⁺ signaling pathways
Increases in cytosolic Ca²⁺ concentrations ([Ca²⁺]ᵢ) occur as a result of: firstly, Ca²⁺ entry from the extracellular space and secondly, Ca²⁺ release from intracellular stores, predominantly from the endoplasmic reticulum (ER).

These sources of Ca²⁺ consist of a large number of Ca²⁺ pumps, channels, exchangers, and Ca²⁺-binding proteins that aim to control intracellular Ca²⁺ levels (Figure 1). Under resting conditions, [Ca²⁺] is maintained at a concentration of approximately 100 nM, whereas extracellular [Ca²⁺] is approximately 1 mM. Ca²⁺ entry is driven by the presence of a large electrochemical gradient across the plasma membrane. Cells manage this external pool of Ca²⁺ by activating various entry channels with widely different properties. The voltage-gated calcium channels (VGCCs), which belong to the Caᵥ family, are activated by depolarizing membrane potentials and are primarily expressed in excitable cells. Otherwise, in non-excitable cells, Ca²⁺ entry mostly occurs through non-voltage-gated channels. These include ligand-gated channels, such as the P2X purinergic ionotropic receptor families [2], and transient receptor potential (TRP) channels, which form a superfamily that is divided into seven subfamilies, the first of which is composed of the ‘canonical’ TRPs (TRPC subfamily) [3]. The main role of the TRP channels is to mediate Ca²⁺ entry in response to various stimuli, including the production of diacylglycerol or stretching of the plasma membrane. Nevertheless, some TRP channels can work as store-operated channels. Indeed, when the ER releases its Ca²⁺ content into the cytosol, a subsequent influx of extracellular Ca²⁺ across membrane channels occurs, which sustains the Ca²⁺ signal and enables the refilling of depleted stores, including the ER [4]. This event is termed SOCE (store-operated Ca²⁺ entry) and is controlled at a molecular level by the canonical TRP channels, the Ca²⁺ release-activated calcium channel protein 1 (ORAI1) and the ER Ca²⁺ sensors STIM1 (stromal interaction molecule 1) and STIM2 [5]. ORAI1 and STIM1 physically interact at ER–plasma membrane junctions in a functional Ca²⁺ release-activated Ca²⁺ (CRAC) channel complex through a dynamic interplay between their helices [6].

ER calcium depletion originates following the binding of physiological ligands to cell surface receptors that activate phospholipase C to produce IP3 (inositol-1,4,5-trisphosphate), a second messenger that mediates the opening of IP3 receptors (IP3Rs) with a consequent rapid release of Ca²⁺ into the cytoplasm. In mammals, different genes encode three isoforms of IP3R, termed IP3R type 1, 2 or 3, which are highly similar in their primary sequences, but differ in terms of regulation [7]. A second family of ER channels, named ryanodine receptors (RyanRs), is involved in Ca²⁺ release. This family contains three members with tissue-specific distributions. The mechanism of activation of each of these isoforms is different, ranging from protein–protein interactions with plasma membrane VGCCs.
Intracellular $\text{Ca}^{2+}$ homeostasis. Various signaling molecules interact with receptors on the plasma membrane and elicit changes in the intracellular $\text{Ca}^{2+}$ concentration. Abbreviations: ER: endoplasmic reticulum; [Ca$^{2+}$]: calcium concentration; CICR $\text{Ca}^{2+}$-induced $\text{Ca}^{2+}$ release; SOCE: store-operated $\text{Ca}^{2+}$ entry; ATP: adenosine triphosphate; AA: arachidonic acid; VGCC: voltage-gated calcium channel; TRP: transient receptor potential channel (C: canonical; V: vanilloid; M: melastatin); ORAI1: $\text{Ca}^{2+}$ release-activated calcium channel protein 1; STIM1: stromal interaction molecule 1; IP3: inositol-1,4,5-triphosphate; IP3R: IP3 receptor; RyR: ryanodine receptor; SERCA: sarco/endoplasmic reticulum $\text{Ca}^{2+}$-ATPase; MCU: mitochondrial calcium uniporter; VDAC: voltage-dependent anion channel; PMCA: plasma-membrane $\text{Ca}^{2+}$-ATPase.

Figure 1

Intracellular $\text{Ca}^{2+}$ homeostasis. Various signaling molecules interact with receptors on the plasma membrane and elicit changes in the intracellular $\text{Ca}^{2+}$ concentration. Abbreviations: ER: endoplasmic reticulum; [Ca$^{2+}$]: calcium concentration; CICR $\text{Ca}^{2+}$-induced $\text{Ca}^{2+}$ release; SOCE: store-operated $\text{Ca}^{2+}$ entry; ATP: adenosine triphosphate; AA: arachidonic acid; VGCC: voltage-gated calcium channel; TRP: transient receptor potential channel (C: canonical; V: vanilloid; M: melastatin); ORAI1: $\text{Ca}^{2+}$ release-activated calcium channel protein 1; STIM1: stromal interaction molecule 1; IP3: inositol-1,4,5-triphosphate; IP3R: IP3 receptor; RyR: ryanodine receptor; SERCA: sarco/endoplasmic reticulum $\text{Ca}^{2+}$-ATPase; MCU: mitochondrial calcium uniporter; VDAC: voltage-dependent anion channel; PMCA: plasma-membrane $\text{Ca}^{2+}$-ATPase.

On the other hand, the sarco/endoplasmic reticulum $\text{Ca}^{2+}$-ATPase (SERCA) pumps catalyze $\text{Ca}^{2+}$ transport into the lumen of the ER through an active process that requires adenosine triphosphate (ATP). Thus, the SERCA system refills the ER $\text{Ca}^{2+}$ content and simultaneously contributes to switching off of $\text{Ca}^{2+}$ signaling. Restoration of basal [Ca$^{2+}$], also occurs via $\text{Ca}^{2+}$ extrusion through the plasma-membrane $\text{Ca}^{2+}$-ATPase (PMCA) and the buffering activity of the mitochondrial compartment [9**]. Upon discharge of ER $\text{Ca}^{2+}$ content, the mitochondria take up a large amount of $\text{Ca}^{2+}$ (10-fold higher than that measured in the cytosol) due to their juxtaposition to the ER, the presence of an electrochemical gradient ($-180 \text{ mV}$) inside the mitochondrial matrix and the activity of a mitochondrial calcium uniporter (MCU) complex [10*]. In addition to $\text{Ca}^{2+}$ buffering functions, mitochondrial $\text{Ca}^{2+}$ accumulation regulates metabolism and cell survival, and its implication in cancer is currently under investigation. Under a variety of stresses or types of damage, excessive $\text{Ca}^{2+}$ is released from the ER through the IP3Rs and transferred to the mitochondria, leading to mitochondrial $\text{Ca}^{2+}$ overload, an opening of the mitochondrial permeability transition pore [11,12] and the release of pro-apoptotic factors into the cytosol.

Thus, both $\text{Ca}^{2+}$ influx and $\text{Ca}^{2+}$ liberation are controlled by a plethora of regulatory systems that provide spatial
and temporal characteristics of an intracellular calcium signal that are required for sustaining specific cellular functions. How alterations of Ca\textsuperscript{2+} homeostasis may impact cancer development will be discussed in the next section.

**Ca\textsuperscript{2+} players directly involved in cancer**

The contribution of Ca\textsuperscript{2+} transporters and channels to tumor development is especially evident in prostate cancer (PCa), which is characterized by enhanced proliferation and apoptosis resistance. Both TRPC6 and TRPV6 (TRP Vanilloid subfamily) display enhanced expression in PCa and are associated with histological grade and Gleason score increases\cite{13,14}. TRPV6 mediates Ca\textsuperscript{2+} entry, which is greatly increased in PCa due to a remodeling mechanism involving the translocation of the TRPV6 channel to the plasma membrane. Moreover, TRPV6-dependent Ca\textsuperscript{2+} influx increases the proliferation of PCa cells and protects them from apoptosis\cite{15}. However, a number of studies have shown that SOCE provides a large, sustained influx of Ca\textsuperscript{2+} that triggers, rather than blocks, apoptosis in cancer cells\cite{16}. Accordingly, the down-regulation of ORAI1, the major molecular component of endogenous SOCE, protects these cells from diverse apoptosis-inducing pathways\cite{17}. Thus, Ca\textsuperscript{2+} entry seems to act as both an inducer and inhibitor of cell death in PCa cells. These discrepant observations can in part be explained in the nature of Ca\textsuperscript{2+} signaling. Indeed, TRPV6 is not a genuine SOC, and Ca\textsuperscript{2+} entry through TRPV6 rapidly inactivates this channel via a negative feedback loop that creates Ca\textsuperscript{2+} transients that contribute to cancer cell survival\cite{18}. Nevertheless, increased expression of ORAI3 (an arachidonic acid-regulated Ca\textsuperscript{2+} channel) or factors in the tumor microenvironment induce the heterodimerization of ORAI1 and ORAI3, which causes a switch from SOCE to arachidonic acid-mediated Ca\textsuperscript{2+} influx that is associated with reduced ORAI1/SOCE-mediated apoptosis and increased arachidonic acid/ORAI3 proliferation and cell migration\cite{19**}. Thus, SOCE-dependent and SOCE-independent mechanisms play different roles in the regulation of apoptotic processes, principally due to the intrinsic functions of Ca\textsuperscript{2+} signaling.

On the other hand, increases in [Ca\textsuperscript{2+}], and pro-metastatic behavior appear to be especially correlated. Several lines of evidence suggest a link between increased proliferation and tumor cell migration together with a higher expression of plasma membrane channels and Ca\textsuperscript{2+} influx, including both TRP channels\cite{20} and P2X receptors\cite{21}. These events have been related to the acquisition of a metastatic cell phenotype\cite{22}. Nonetheless, Ca\textsuperscript{2+} mobilization from the ER also contributes to cell migration, and aberrant increases in IP3Rs levels have been observed in different metastatic tumors. In particular, IP3R type 3 (IP3R3) has been shown to be involved in breast cancer proliferation\cite{23}, and elevated expression levels correlate with enhanced invasion and metastasis and decreased long-term survival in colorectal cancer\cite{24}, as well as the dissemination of gastric cancers\cite{25}. However, IP3R3 has been demonstrated to be the preferential isoform that conveys apoptotic Ca\textsuperscript{2+} signals to the mitochondria\cite{26} and to contribute actively to cell death in a variety of tissues\cite{27}.

Overall, these findings suggest a model depicting a multi-phase Ca\textsuperscript{2+} remodeling in tumor cells, in which increases of [Ca\textsuperscript{2+}], promote cell migration and represent an important factor in the metastatic behavior of cancer cells, whereas reduced ER-mitochondria Ca\textsuperscript{2+} transfer and/or attenuation of SOCE modulate cell death, thus actively contributing to acquired resistance to apoptosis of primary tumors. Notably, recent analyses of head and neck squamous cell carcinoma samples identified IP3R3 missense mutations in multiple nodal metastases, but not in the primary tumors, which may confer metastatic ability\cite{28*}.

Therefore, considering the pivotal role played by Ca\textsuperscript{2+} in the control of cancer dynamics, it appears likely that the various oncogenes and tumor suppressors that are most often altered in cancer cells can influence Ca\textsuperscript{2+} signaling to exert their pro-oncogenic or anti-oncogenic functions.

**Oncogenes and tumor suppressors regulating Ca\textsuperscript{2+} signaling**

Historically, Bel-2 was the first oncogene to be linked to a Ca\textsuperscript{2+}-dependent cancer activity. At the ER, Bel-2 lowers the steady-state ER Ca\textsuperscript{2+}-store content, thus protecting mitochondria from Ca\textsuperscript{2+} overload\cite{29,30}. Different mechanisms have been proposed to explain this effect, including a role for Bel-2 as a Ca\textsuperscript{2+} leak channel and a modulator of SOCE\cite{29}, or Bel-2-dependent inactivation of SERCA\cite{31}. Conversely, Bel-2 has also been described as an inhibitor of both IP3Rs\cite{32,33} and RyRs\cite{34} functions. Alteration of Ca\textsuperscript{2+} homeostasis is a common feature of each of the anti-apoptotic Bel-2 family members. Both Bel-XL-mediated and Mcl-1-mediated apoptosis resistance is afforded by the interaction of each IP3R isoform through a mechanism involving enhanced low-level [Ca\textsuperscript{2+}] signaling\cite{35,36}. Similar to Bel-2, Bel-XL may also directly bind to RyRs\cite{37}.

During the past 5 years, our group has described a detailed molecular process that takes place at Mitochondria Associated Membranes (MAMs)\cite{38}, involving the IP3R3, Akt and the tumor suppressors PML (promyelocytic leukemia protein) and PTEN (phosphatase and tensin homolog deleted on chromosome 10). Akt-dependent phosphorylation of IP3R3 inhibits ER Ca\textsuperscript{2+} efflux, conferring resistance to apoptosis\cite{39}. Both PTEN and PML localization at MAMs reduce Akt activity and rescue susceptibility to cell death through direct dephosphorylation/inactivation of Akt (PTEN)\cite{40} or by promoting the formation of a multiprotein complex containing IP3R3, Akt, and the protein phosphatase PP2a (PML)\cite{41}. These findings
have been recently confirmed by the identification of a specific distribution of the Akt activator mTORc2 (mechanistic target of rapamycin complex 2) at MAMs [42]. Moreover, several onco-suppressors show anti-cancer activities linked to the rearrangement of \(Ca^{2+}\) dynamics. These include the modulation of mitochondrial \(Ca^{2+}\) uptake by FHIT (fragile histidine triad) [43], the induction of IP3R-mediated apoptotic \(Ca^{2+}\) release by BRCA1 (breast and ovarian cancer susceptibility gene 1) [44] and a non-transcriptional role for p53, which interacts with SERCA and changes its oxidative state, thus leading to an increased \(Ca^{2+}\) load and consequent mitochondrial damage [45].

Overall, these observations show that the deregulation of \(Ca^{2+}\) signaling by oncogene or tumor-suppressor activities is often associated with cell transformation as it allows tumor cells to escape from apoptosis [46]. However, only a few studies provide evidence that links \(Ca^{2+}\) alterations by oncoprotein expression to cellular migration and invasion during tumor progression. It has been shown that Mcl-1 promotes lung cancer cell migration by binding to the mitochondrial outer membrane-localized voltage-dependent anion channel (VDAC) and allowing for mitochondrial \(Ca^{2+}\) entry and Reactive Oxygen Species (ROS) production [47]. In addition, we recently reported that Mcl-1 tightly controls different mitochondrial parameters, including \(Ca^{2+}\) uptake and morphology [48].

Conversely, several lines of evidence connect micro RNAs (miRs) and pro-metastatic features through the remodeling of intracellular \(Ca^{2+}\) levels. In patients with breast cancer, miR-708 expression was decreased in distal metastases, suggesting a metastasis-suppressive role. miR-708 targets the ER protein neuronatin, lowering intracellular \(Ca^{2+}\) fluxes and impairing the metastatic potential of breast cancer cells [49]. Again, a single nucleotide polymorphism in the 3′-UTR of the \(RYR3\) gene, which prevents binding of miR-367, causes elevated \(RyR3\) expression in patient samples and the induction of breast cancer cell growth, aberrant morphology, and migration [50]. Finally, the expression of miR-185 inversely correlates with the expression of STIM1 in colorectal cancer (CRC) cells and is associated with poor differentiation and higher tumor node metastasis staging [51]. Interestingly, human CRC samples also displayed high levels of miR-25, which reduces mitochondrial \(Ca^{2+}\) entry and protects from apoptosis by targeting MCU [52].

**Concluding remarks**

The deregulation of \(Ca^{2+}\) homeostasis is an important factor in the metastatic behaviors of cancer cells and in conferring tumor resistance to apoptosis [53]. Alterations of \(Ca^{2+}\) signaling occur in a wide range of tumors, including malignant pleural mesothelioma [54], and may contribute to the inefficacy of some chemotherapeutic agents. Indeed, measurements of intracellular \(Ca^{2+}\) dynamics \textit{in vivo} within tumor masses have shown that phthalocyanine, a light-activatable agent used in cancer photodynamic therapy, exhibits reduced activity upon the inhibition of \(Ca^{2+}\) signals, such as in the presence of the \(Ca^{2+}\)-chelator BAPTA or when ER-mitochondria \(Ca^{2+}\) transfer is impaired [55]. Moreover, the chemotherapeutic compound AECHL-1, which belongs to triterpenoids, a group of small molecules with demonstrated anticancer activities in preclinical models and in clinical trials, exerts its anti-neoplastic activity in a \(Ca^{2+}\)-dependent manner [56]. Therefore, an accurate analysis of \(Ca^{2+}\) signaling in different tumor contexts is required to optimize the activity of some anti-cancer agents and to develop a \(Ca^{2+}\)-based pharmacological approach for the treatment of cancer.

**Conflict of interest statement**

Nothing declared.

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**References and recommended reading**

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

This review describes the molecular characterization of the recently discovered MCU complex.


This study demonstrates the importance of an oncogenic remodelling of Ca2+ influx which occurs in prostate cancer cells.


Identification of IP3R type 3 cancer-related mutations.


42. Betz C, Stracka D, Prescianotto-Baschong C, Frieden M, Denauxre N, Hall MW: Feature article: tMOR complex 2-Akt signalling at mitochondria-associated endoplasmic reticulum.


This study reveals a novel, non-transcriptional and Ca2+-related function of cytosolic p53 in cell death.


