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Calcium wave signaling in cancer cells

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Abstract

Ca²⁺ functions as an important signaling messenger right from beginning of the life to final moment of the end of the life. Ca²⁺ is needed at several steps of the cell cycle such as early G₁, at the G₁/S, and G₂/M transitions. The Ca²⁺ signals in the form of time-dependent changes in intracellular Ca²⁺ concentrations, [Ca²⁺]_i, are presented as brief spikes organized into regenerative Ca²⁺ waves. Ca²⁺-mediated signaling pathways have also been shown to play important roles in carcinogenesis such as transformation of normal cells to cancerous cells, tumor formation and growth, invasion, angiogenesis and metastasis. Since the global Ca²⁺ oscillations arise from Ca²⁺ waves initiated locally, it results in stochastic oscillations because although each cell has many IP₃Rs and Ca²⁺ ions, the law of large numbers does not apply to the initiating event which is restricted to very few IP₃Rs due to steep Ca²⁺ concentration gradients. The specific Ca²⁺ signaling information is likely to be encoded in a *calcium code* as the amplitude, duration, frequency, waveform or timing of Ca²⁺ oscillations and decoded again at a later stage. Since Ca²⁺ channels or pumps involved in regulating Ca²⁺ signaling pathways show altered expression in cancer, one can target these Ca²⁺ channels and pumps as therapeutic options to decrease proliferation of cancer cells and to promote their apoptosis. These studies can provide novel insights into alterations in Ca²⁺ wave patterns in carcinogenesis and lead to development of newer technologies based on Ca²⁺ waves for the diagnosis and therapy of cancer.

Keywords

Apoptosis; Calcium; Cancer; Cell cycle; Metastasis; Oscillations; Signaling; Stochastic; Wave

Introduction

At the beginning of life, Ca²⁺ mediates the process of fertilization as well as regulates the cell cycle events during the early developmental processes. Once the cells differentiate to perform specific functions, Ca²⁺ regulates several processes as diverse as energy

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transduction, secretions, apoptosis, muscle contraction, chemotaxis and neuronal synaptic plasticity in learning and memory (Berridge 2009; Berridge et al. 2003; Clapham 2007; Mikoshiba 2007). Since Ca^{2+} ions are toxic to the cells, the intracellular concentration of Ca^{2+} , $[\text{Ca}^{2+}]_i$, in resting cells is usually maintained very low at ~ 100 nM. While in normal salt solution, the hydrated Ca^{2+} can diffuse $40\text{ }\mu\text{m}$ in 1 sec (Einstein 1905); in cells, due to the presence of several charged molecules, the Ca^{2+} diffusion rates are slower. In order to utilize Ca^{2+} as a second messenger, cells have devised an ingenious mechanism of signaling that has overcome the inherent problems associated with lower diffusion rates and cytotoxicity of Ca^{2+} , by presenting changes in Ca^{2+} concentration as brief spikes which are often organized as regenerative waves (Berridge 1993; Clapham 2007; Rey et al. 2010).

To provide for a very fast and effective Ca^{2+} -signaling, the cells use a great amount of energy to maintain almost 20 000-fold Ca^{2+} -gradient between their intracellular (~ 100 nM free) and extracellular (~ 1.2 mM) Ca^{2+} concentrations. To maintain this 20 000-fold Ca^{2+} gradient, the cells chelate, compartmentalize, or remove Ca^{2+} from the cytoplasm (Clapham 2007). Various cellular proteins with Ca^{2+} -binding affinities ranging between nM to mM are utilized by the cells to buffer the cellular Ca^{2+} as well as to regulate cellular processes via Ca^{2+} -signaling (see Fig. 1). The binding of Ca^{2+} to proteins can change protein conformations in terms of shape and charge, and thus modify its functions (Westheimer 1987). Ca^{2+} -mediated signaling pathways have been shown to play important roles in cancer initiation, tumor formation, tumor progression, metastasis, invasion and angiogenesis (Block et al. 2010; Rizzuto and Pozzan 2006; Saidak et al. 2009). Some of the Ca^{2+} -regulated pathways involved in cancers are briefly summarized in BOX 1.

Both the genetic and epigenetic mechanisms have been proposed for the specific roles of Ca^{2+} signaling in cancer (Hanahan and Weinberg 2000; Jaffe 2005). As a result of mutations, the normal cells can be transformed to cancerous cells by acquiring cancer-specific properties such as uncontrollable proliferation, immortality, and self-sufficiency in growth signals (Futreal et al. 2005; Hanahan and Weinberg 2000). Since Ca^{2+} -gradients and waves have been shown to be involved in normal developmental processes, the epigenetic theory proposes that altered Ca^{2+} -gradients and waves participate in carcinogenesis (Jaffe 2005). The intracellular Ca^{2+} waves in concert with various other signal-transduction cascades, regulate a variety of processes including gene expression (De Koninck and Schulman 1998; Dolmetsch 2003; Dolmetsch et al. 1998). The intracellular Ca^{2+} -induced activation of protein kinase C (PKC) can lead to phosphorylation of methyltransferase that is involved in DNA methylation (DePaoli-Roach et al. 1986). In normal cells, Ca^{2+} -signaling is required for cell proliferation, whereas some transformed cells and tumor cell lines show altered dependency on Ca^{2+} to maintain proliferation (Cook and Lockyear 2006; Whitfield 1992). Ca^{2+} -signaling pathways are remodeled or deregulated in cancer that result in changes in their physiology and distinguish them from non-malignant cells (Hanahan and Weinberg 2000; Wang et al. 2010). Remodeling or deregulation of Ca^{2+} -signaling pathways can provide means by which cancer cells can overcome systemic anticancer defense mechanisms. The altered Ca^{2+} -signaling pathways can also lead to genetic diversity found in cancerous tissues thereby providing effective cellular strategies to the selection pressure to acquire specific traits.

In this review, we first describe our present understanding of Ca^{2+} -signaling in cells including Ca^{2+} fluxes across the plasma membrane, Ca^{2+} translocation across the ER, wave nature of Ca^{2+} signals, and the role of various Ca^{2+} binding proteins followed by extensive discussion on the role of Ca^{2+} signaling in cancer cells proliferation and apoptosis (Berridge 2005; Carafoli 2004; Petersen 2005; Rizzuto and Pozzan 2006).

Box 1: Ca^{2+} and cancer

The cells maintain a 20 000-fold gradient of Ca^{2+} between extracellular free Ca^{2+} (~ 1.2 mM) and resting cytoplasmic free Ca^{2+} (~ 100 nM). Depending upon the stimulus, $[\text{Ca}^{2+}]_i$ can exceed more than 1 μM (Rizzuto and Pozzan 2006; Rizzuto et al. 2003). The spatio-temporal nature of changes in the free Ca^{2+} can regulate many cellular processes including cancer initiation, progression, angiogenesis, and metastasis as described below (Rizzuto and Pozzan 2006; Rizzuto et al. 2003; Saidak et al. 2009):

Transcription

The changes in Ca^{2+} wave oscillation frequency can activate transcription factors such as nuclear factor of activated T cells (NFAT) resulting in modulation of cellular transcription (Carafoli 2002; Dolmetsch 2003; Rizzuto and Pozzan 2006). Experiments using the “calcium clamp” have shown that the sensitivity of different transcription factors to $[\text{Ca}^{2+}]_i$ oscillations is highly frequency dependent (Dolmetsch et al. 1997; Lewis 2003).

Cell cycle

Since Ca^{2+} regulates the cell cycle at several stages, it is involved in cell proliferation through various signaling pathways (Cullen and Lockyer 2002; Ding et al. 2010; Minaguchi et al. 2006; Rey et al. 2010; Zhong et al. 2010). The Ca^{2+} -induced activation of the transcription of early genes is involved in the transition from G_0 to G_1 . The subcellular localization of key proteins associated with cell cycle and carcinogenesis is influenced by Ca^{2+} (Cook and Lockyer 2006). Ca^{2+} has been shown to regulate the phosphorylation of retinoblastoma protein in late G_1 phase (Cook and Lockyer 2006).

Genotoxicity

Ca^{2+} can modulate poly-(ADP-ribose) polymerase-1 (PARP1) as well as DNA damage response pathways which in turn can influence genomic stability and cell survival (Bentle et al. 2006).

Differentiation

Cancer cells can become dedifferentiated into cancer stem cells in the tumorigenic process, and Ca^{2+} signaling is implicated in the differentiation process either through the extracellular Ca^{2+} -sensing receptor and/or through alterations in intracellular Ca^{2+} (Bikle et al. 2004).

Angiogenesis

By mobilizing release of Ca^{2+} from ER, angiogenic factors such as vascular endothelial growth factor can increase $[\text{Ca}^{2+}]_i$ and therefore induce Ca^{2+} signaling that in turn plays critical roles in angiogenesis (Munaron and Fiorio 2009; Patton et al. 2003).

Telomerase

Cancer cells upregulate telomerase expression in order to maintain their telomeres for cellular immortality. The Ca^{2+} binding protein S100A8 can inhibit the activity of telomerase (Rosenberger et al. 2007).

Motility

Ca^{2+} has been associated with motility for a long time since Sidney Ringer found that it was the Ca^{2+} in London's “hard” tap water that was required for heart contraction (Ringer 1883).

The intracellular Ca^{2+} signaling plays important role in cellular motility such as during tumor invasion and metastasis, the extracellular Ca^{2+} signaling has been shown to be associated with bone metastasis (Amuthan et al. 2002; Berridge et al. 2003; Huang et al. 2004; Zhong et al. 2010).

Apoptosis

The increase in $[\text{Ca}^{2+}]_i$ and $[\text{Ca}^{2+}]_{\text{mito}}$ and the activation of mitochondrial membrane permeabilization can lead to apoptosis and necrosis (Rizzuto and Pozzan 2006; Rizzuto et al. 2003). Similarly, a decrease in the Ca^{2+} content of the lumen of the ER is associated with resistance to apoptosis (Rizzuto et al. 2003; Pinton and Rizzuto 2006).

Ca^{2+} signaling initiation

In order to maintain low $[\text{Ca}^{2+}]_i$, the ATPase pump such as sarcoendoplasmic reticular Ca^{2+} ATPase (SERCA) pumps in Ca^{2+} into ER by exchanging protons for two Ca^{2+} per ATP hydrolyzed whereas plasma membrane Ca^{2+} -ATPase (PMCA) removes Ca^{2+} by exchanging protons for 1 Ca^{2+} per ATP hydrolyzed (Figure 1). In addition, the $\text{Na}^+/\text{Ca}^{2+}$ exchangers (NCX), and the $\text{Na}^+/\text{Ca}^{2+}$ - K^+ exchangers (NCKX) exchange one Ca^{2+} ion for three Na^+ ions or cotransport one K^+ ion with one Ca^{2+} ion in exchange for four Na^+ ions, respectively. In its “forward” mode, inward (depolarizing) Na^+ current drives Ca^{2+} extrusion from the cell. While the PMCA is effective in maintaining low $[\text{Ca}^{2+}]_i$ over longer durations, NCX and NCKX function at a much faster rate to maintain low $[\text{Ca}^{2+}]_i$ (Hilgemann et al. 2006). Transient receptor potential (TRP) ion channels formed by tetrameric assembly around a pore, are weakly voltage-sensitive, nonselective ion channels (Goswami et al. 2010; Ramsey et al. 2006). TRP channels depolarize cells and increase intracellular Na^+ and Ca^{2+} . TRP channels are greatly potentiated by phospholipase C (PLC) activation by G protein-coupled receptors (GPCRs) or tyrosine-kinase receptors (TKR).

The voltage dependent Ca^{2+} channels (CaVs) are the fastest Ca^{2+} signaling proteins and each CaV channel conducts approximately one million Ca^{2+} ions per second down the 20 000-fold gradient therefore a few thousand CaV channels/cell can increase $[\text{Ca}^{2+}]_i$ by more than 10 fold within milliseconds (Fig.1). A change in membrane potential opens the CaV “gate” and allows Ca^{2+} to move into the cytoplasm. In the presence of normal extracellular $[\text{Ca}^{2+}]_o$, the binding of Ca^{2+} to CaV provides ion selectivity to enter the pore (Goux and Mackinnon 2005; Long et al. 2005). However, when external Ca^{2+} is removed, these Ca^{2+} channels become nonselective and allow the entry of Na^+ and K^+ across the cell membrane. The increases in $[\text{Ca}^{2+}]_i$ can initiate translocation of several proteins, e.g., protein kinase C family proteins to specific regions of membranes. Another Ca^{2+} -dependent membrane targeting scheme is used by annexins, where phosphoryl moieties of the membrane replace charge from carbonyl oxygens and water in a unique Ca^{2+} -binding fold (Gerke et al. 2005).

The highly negatively charged phosphatidylinositol 4, 5-bisphosphate (PIP2) is bound to inner leaflets of plasma membranes by its acyl chains (see Fig.1). The positively charged residues on many peripheral and integral membrane proteins can attract negatively charged PIP2, leading to increase in the local concentration of PIP2 that attracts the positively charged proteins cluster close to the membrane. The negatively charged calmodulin present in the cytoplasm competes with PIP2 and may pull the positively charged protein segment off the membrane (McLaughlin and Murray 2005).

A universal mechanism for Ca^{2+} signaling is the release of Ca^{2+} from intracellular Ca^{2+} stores such as ER or SR (Fig.1). G-protein coupled receptors (GPCRs), primarily Gq/11 subtypes can activate phospholipase C β (PLC β) and tyrosine kinase receptors (TKR) can activate PLC γ which then cleave PIP2 into 1,4,5-inositol trisphosphate (IP_3) and

diacylglycerol (DAG). IP₃ binding to the IP₃ receptors (IP₃R) that are present in the ER causes efflux of Ca²⁺ from the ER to the cytoplasm resulting in increase in [Ca²⁺]_i from ~100 nM to ~1 μM for several seconds (Berridge 2009; Mikoshiba 2007). The binding of Ca²⁺ to the C2 domain of PKC α, β1, β2, and γ subtypes initiates translocation to the membrane, where coincident DAG binding activates it. Ca²⁺-sensitive DAG kinase phosphorylates DAG to produce phosphatidic acid, while DAG lipase converts DAG to arachidonic acid. (Fig. 1). IP₃R mediated efflux of Ca²⁺ from the ER in response receptor activation empties the ER as PMCAs pump Ca²⁺ out of the cell faster than it can be replenished. Slowly over seconds after such store depletion, a Ca²⁺ entry mechanism is activated. This mechanism is called store-operated Ca²⁺ entry (Putney 2005). A slow and tiny but highly selective Ca²⁺ conductance is activated when ER [Ca²⁺] drops and it is called Ca²⁺-release activated current (I_{CRAC}) (Parekh and Penner 1997).

IP₃Rs are nonselective cationic channels that conduct Ca²⁺. The IP₃R complex is a massive tetrameric channel with its pore formed by a homotetramers of ~3,000 amino acids. The six transmembrane-spanning domain is at the very C-terminal end of each subunit, that provides for a large number of cytoplasmic regulatory sites and protein-binding domains. Regulated by an intrinsic suppressor domain, a hinged clamshell-like structure clasps IP₃. Many proteins such as IP₃R-binding protein (IRBIT) released with IP₃ are proposed to interact with the IP₃R whereas binding of ERp44 confers redox sensing (Mikoshiba 2007). Like the IP₃R, ryanodine receptors (RyRs) are also massive tetrameric (2.2 mDa) channels permeant to Ca²⁺ that span the ER or SR. The primary natural agonist of RyR is Ca²⁺, low [Ca²⁺]_i opens the channel to allow Ca²⁺ to flow out of the ER/SR whereas higher [Ca²⁺]_i near the openings of channel inhibits Ca²⁺ gating and thus prevents [Ca²⁺]_i overload. Approximately 80% of the RyR's mass is located towards cytoplasm, where it interacts with several proteins such as Ca/CaM/CaMKII, FK-506-binding proteins, mAKAP/PKA, PR130/calcineurin, spinophilin, and sorcin. Several other proteins such as triadin, junctin, and calsequestrin also regulate ER Ca²⁺ availability to the pore (Bers 2004). IP₃Rs and/or RyRs are also sensitive to the redox status of cells, to nitric oxide/S-nitrosylation, and to quinones/reactive oxygen species (Waring 2005).

Calcium waves in signaling

The release of Ca²⁺ from the ER is a nonlinear, cooperative process wherein IP₃ binds to four receptor sites on the IP₃R, one on each subunit of the tetramer (Mikoshiba 2007). In both ER and SR, IP₃Rs and RyRs are at first potentiated, then inhibited by Ca²⁺. Small perturbations in conditions, such as ambient [Ca²⁺]_i, [IP₃], and various regulators can cause uncoordinated bursts of local release across a cell (called “sparks” for their appearance in Ca²⁺ imaging fluorescence microscopy (see Fig. 2; Guatimosim et al. 2002). The brief opening of these channels gives rise to localized pulses (Fig. 2) such as the sparks or blips and puffs (Cheng et al. 1993; Parker and Yao 1996; Yao et al. 1995). The appearance of sparks or blips and puffs results in increase in the [Ca²⁺]_i (Bootman and Berridge 1996; Parker and Yao 1996). At higher levels of IP₃, the blips grow into larger puffs that can act as initiation sites for the onset of Ca²⁺ waves (Fig. 2). If the IP₃R are sufficiently sensitized, they will respond to the Ca²⁺ diffusing away from a puff site and thereby propagate the signal through a process of calcium-induced calcium release (CICR) as depicted in Fig. 2. These waves are the mechanisms that coordinate the release of Ca²⁺ by all the IP₃R. Therefore, increasing spark frequency can cascade and become regenerative and is seen as two- or three dimensional waves of changes in [Ca²⁺]_i that propagate within cells (Fig. 2). These Ca²⁺ waves can interact with each other and create spirals or annihilate each other. The information transmitted by these Ca²⁺ waves can arrive as a stimulus at the plasma membrane and is translated into intracellular Ca²⁺ oscillations (Falcke 2004).

The smallest Ca^{2+} release events, blips (Parker et al. 1996), probably reflect random openings of single IP_3R (Fig. 2). Larger events, puffs, lasting tens of milliseconds and restricted to a volume of ~ 0.5 fl, reflect the almost simultaneous opening of a few IP_3Rs within a cluster (Parker et al. 1996; Suhara et al. 2006). Several coordinated puffs can form oscillations and waves as shown in Fig. 2 (Falcke 2004). Ca^{2+} oscillations, therefore, depend upon both the spatial organization of IP_3Rs and their regulation by Ca^{2+} although the links between IP_3R activities and Ca^{2+} oscillations are not fully understood (Taylor and Laude 2002).

How are random molecular events such as blips and puffs orchestrated into global intracellular Ca^{2+} oscillations? It is generally assumed if many molecules are involved in these processes, the cell behaves like a continuously stirred reactor and the law of large numbers can be used to predict such Ca^{2+} oscillations (van Kampen 2001). Though most models of the dynamics of intracellular Ca^{2+} release use the law of large numbers yet it is not consistent with experimental analyses showing that global oscillations arise from Ca^{2+} waves initiated locally (Falcke 2004; Marchant and Parker 2001). Therefore, such a local initiation of Ca^{2+} waves is predicted to lead to stochastic oscillations because although each cell has many IP_3Rs and Ca^{2+} ions, the law of large numbers does not apply to the initiating event which is restricted to very few IP_3Rs due to steep Ca^{2+} concentration gradients (Falcke 2003, 2004).

The stochastic nature of the release of Ca^{2+} from internal calcium stores such as ER into the cytoplasm have been studied by several investigators (Cheng et al. 1993). All ion channels have certain properties such as open or closed, conducting or non-conducting, with opening and closing describable as Markov processes. The stochastic nature of Ca^{2+} release is nicely shown by the observed onset of whole-cell Ca^{2+} oscillations as $[\text{IP}_3]$ is increased in *Xenopus* oocytes (Marchant and Parker 2001). While at low $[\text{IP}_3]$, only puffs are observed because there is not enough Ca^{2+} released from a cluster to stimulate Ca^{2+} release from neighboring sites, therefore the Ca^{2+} transient is purely local (see Fig. 2). At moderately higher $[\text{IP}_3]$, the amount of Ca^{2+} released from each IP_3R increases, leading to formation of Ca^{2+} waves that emerge from a nucleation site. However, at moderate levels of $[\text{IP}_3]$, global events are rare and in many cases there are waves that progress only a short distance before dying out. However, at very high $[\text{IP}_3]$, global Ca^{2+} waves are seen to occur regularly with a well-defined period.

The changes in the cytoplasmic Ca^{2+} can be defined as follows (Falcke 2004):

$$d[\text{Ca}^{2+}]_i/dt = J_{\text{rel}} - J_{\text{uptake}}, \quad (\text{eq. 1})$$

where $[\text{Ca}^{2+}]_i$ is the cytoplasmic concentration of Ca^{2+} , J_{rel} is the Ca^{2+} flux (in units of concentration per unit time) into the cytoplasm through release from calcium stores and J_{uptake} is the flux of Ca^{2+} via uptake into the calcium stores. The Ca^{2+} release flux J_{rel} can be described by the following equation:

$$J_{\text{rel}} = gPo \left([\text{Ca}^{2+}]_{\text{ER}} - [\text{Ca}^{2+}]_i \right), \quad (\text{eq. 2})$$

where Po is the open probability, g is the maximal conductance, and the Ca^{2+} channel driving force $[\text{Ca}^{2+}]_{\text{ER}} - [\text{Ca}^{2+}]_i$ is difference between ER calcium $[\text{Ca}^{2+}]_{\text{ER}}$ and cytoplasmic calcium $[\text{Ca}^{2+}]_i$. To account for the stochastic and spatial nature of the receptors, a more accurate model can be described by the following equation:

$$\partial[Ca^{2+}]_i/\partial t = D\nabla^2[Ca^{2+}]_i + \sum_k \delta(x - x_k) J_{rel}^k - J_{uptake}, \quad (\text{eq. 3})$$

where J_{rel}^k represents the release of calcium from the k th release unit and x_k represents the spatial location of single channels or small clusters of channels. J_{rel}^k can be described by the following equation:

$$J_{rel}^k = g_k p_k \left([Ca^{2+}]_{ER} - [Ca^{2+}]_i \right), \quad (\text{eq. 4})$$

where p_k is a random variable with values 0 or 1 to indicate whether the release unit is closed or open. In presence of a very large number of Ca^{2+} release sites, p_k can be replaced by the average open probability P_o and thus obtain the whole-cell model in the limit that diffusion is large compared to the total size of the cell. However, these assumptions are not valid in many physiological situations, such as in oocytes and pancreatic beta cells (Sherman and Rinzel 1991). Although it is possible to use Monte Carlo simulations to describe the random behavior of Ca^{2+} release events, such computations may not provide correct answers in multicellular settings (Bugrim et al. 1997; Falcke 2003).

Cell-to-Cell Signaling

The Ca^{2+} signaling among cells can occur in two ways. In the first process, the cells can use gap junction (connexin) channels to communicate with one another (Rossello et al. 2009). In the second process, the cell-to-cell signaling is performed by transmitter-gated Ca^{2+} permeant ion channels such as NMDA, nicotinic, purinergic ionotropic channels. The opening of CaV can rapidly increase the local periplasmic $[Ca^{2+}]$ resulting in triggering of protein-fusion complexes such as synaptotagmins and SNARE complexes, and fusion of vesicles containing transmitter molecules with the plasma membrane. These transmitter molecules such as ATP, acetylcholine, or glutamate that are released outside the cell could gate Ca^{2+} -permeant channels namely P2X, nicotinic receptors, and NMDA receptors on adjacent cell membranes (Block et al. 2010; Jahn and Scheller 2006; Rossello et al. 2009; Sudhof 2004).

Remodeling of Ca^{2+} signaling in cancer cell proliferation

Ca^{2+} plays important role throughout the mammalian cell cycle such as in early G_1 , at the G_1/S , and G_2/M transitions (Fig. 3). The expression of immediate-early genes in G_1 , such as *FOS*, *JUN* and *MYC*, and the retinoblastoma (RB1) phosphorylation at the G_1/S boundary are regulated by Ca^{2+} (Pande et al. 1996; Takuwa et al. 1996). Similarly, the Ca^{2+} binding protein calmodulin (CaM) plays crucial roles in cell cycle progression through G_1 and mitosis (Kahl and Means 2003; Rasmussen and Means 1989). It has been shown that the cells can be arrested in G_1 stage by inhibition of CaM kinase (CaMK) leading to loss of cyclin D1 (CCND1) expression, and inhibition of cyclin-dependent kinase 4 (CDK4) and CDK2 (Fig. 3). Another target of CaM, calcineurin also plays a major role in the cell cycle progression through G_1 and S phases by regulating cAMP-responsive element binding protein 1 (CREB1) and NFAT (Kahl and Means 2004). In its inactive phosphorylated state, NFAT localizes in the cytoplasm, but following an increase in $[Ca^{2+}]_i$, the activated calcineurin dephosphorylates NFAT, which then translocates to the nucleus and regulates expression of its target genes (see Fig. 4). The influx of Ca^{2+} through plasma membrane channels such as the store-operated channel ORAI1 as well as efflux of Ca^{2+} from the ER can activate calcineurin and NFAT signaling pathway for inducing changes in cell cycle gene expression (Fig. 4; Gwack et al. 2007).

In prostate cancer, it has been shown that the increased expression of TRPV6, a constitutively active Ca^{2+} channel, resulted in increased Ca^{2+} entry and therefore enhanced NFAT activation (Lehen'kyi et al. 2007). TRPV6 expression in high-grade prostate cancer can therefore act as biomarker of prognosis (Fixemer et al. 2003). Similarly, the overexpression of a constitutively active NFATC1 mutant in 3T3-L1 fibroblasts induced the expression of MYC, cyclins D1 and D2 (CCND2) resulting in a transformed phenotype (Buchholz et al. 2006). Since cyclin E and E2F are transcriptional targets of MYC, the NFAT-MYC signaling pathway connecting calcineurin and Ca^{2+} is crucial in cell cycle and provides for a very important role of Ca^{2+} (see Fig. 4).

Although the Ca^{2+} -dependent signaling mechanisms are frequently remodeled or deregulated in cancer cells, the lack of studies showing mutations in genes associated with the Ca^{2+} regulating proteins may indicate that remodeling of Ca^{2+} signaling could arise due to epigenetic changes in gene expression and/or post-translational changes (Table 1) in the properties of existing signaling components (Monteith et al. 2007; Saidak et al. 2009).

Remodeling of Ca^{2+} signaling in cancer cell survival and death

Several studies have shown higher levels of $[\text{Ca}^{2+}]_i$ in activation and execution of cell death (Szado et al. 2008). However, through increased expression of pro-survival signaling proteins such as the anti-apoptotic BCL2 family members and suppression of death signaling pathways, the cancer cell can survive in presence of death-inducing stimuli (Edinger and Thompson 2004; Hanahan and Weiberg 2000).

By virtue of regulating the levels of $[\text{Ca}^{2+}]_i$, the two organelles namely ER and mitochondria play crucial roles in Ca^{2+} signaling and deciding the ultimate fate of the cell. The same Ca^{2+} efflux from ER that is responsible for regulating processes for maintaining life, could act as a death-inducing signal at higher efflux of Ca^{2+} (Scorrano et al. 2003). It has been shown that in cells in which IP_3R expression was either reduced or completely silenced, showed significantly less apoptosis (Jayaraman and Marks 1997). Similarly, the decrease in the level of basal IP_3 also prevented cell death (Szado et al. 2008). The higher levels of $[\text{Ca}^{2+}]_i$ resulting due to increased efflux of Ca^{2+} from the IP_3R activity, in turn can cause cell death through cytochrome c binding to IP_3R or due to the activation of caspases (Aseefa et al. 2003). Similarly the activation of RyRs can generate increased levels of cytoplasmic Ca^{2+} that can participate in cell apoptosis (Hajnoczky et al., 2000). The targeting of mitochondrial networks by the proximity of Ca^{2+} signals arising from the ER can play important functions in cell death (Csordas et al., 2006).

In order for the cancer cells to proliferate at higher rates and still protect themselves from apoptosis, the cancer cells need to remodel the Ca^{2+} signaling machinery. Since ER and mitochondria play significant roles in the regulation of cell proliferation and apoptosis, the remodeling of Ca^{2+} signaling machinery in ER and mitochondria in cancer cells seems imminent during oncogenic transformation. In addition, in the cancer cells the increased expression of anti-apoptotic members of the BCL2 family of proteins, or decreased expression of the pro-apoptotic BH3-only proteins or BAX or BAK can protect these cells from apoptosis by modulating intracellular Ca^{2+} signals (Rong and Distelhorst 2008; Scorrano et al. 2003). It has been shown that BCL2 family of proteins can decrease the level of Ca^{2+} fluxes from the ER either by binding to IP_3R or by decreasing the Ca^{2+} content in the lumen of ER through inhibition of SERCA2 (Chen et al. 2004). In addition BCL2 can decrease the sensitivity of the mitochondrial Ca^{2+} uptake process as well as increasing their capacity to accumulate more Ca^{2+} (Danial and Korsmeyer 2004). Scorrano et al. (2003) have shown decreased level of ER Ca^{2+} and Ca^{2+} signals in apoptosis-resistant *Bax*- and *Bak*-knockout mouse embryonic fibroblasts.

Alterations in Ca²⁺ channels and pumps in cancer

Since Ca²⁺ channels or pumps involved in regulating Ca²⁺ signaling pathways show altered expression in cancer, one can target these Ca²⁺ channels and pumps as therapeutic options to downregulate proliferation of cancer cells and enhance their apoptosis. Table 1 shows a comprehensive list of Ca²⁺ transport proteins such as specific isoforms of active transport Ca²⁺ pumps such as the PMCA and the SERCA and Ca²⁺ channels, including voltage-gated and those of the TRP class and ATP-activated P2X ion channel family that are altered in cancer. The altered expression or activity of Ca²⁺ channels and pumps could either cause or promote cancers through modulation of Ca²⁺ concentrations in cytoplasm, ER/SR, and mitochondria as well as spatio-temporal nature of Ca²⁺ signaling (Berridge et al. 2003; Berridge 2009; Mikoshiba 2007). The overexpression of proteins such as IP₃R Ca²⁺ release channels that can cause an increase in Ca²⁺ leakage from the ER, thereby reducing the Ca²⁺ content of the ER Ca²⁺ stores or reduced sequestration of Ca²⁺ as a consequence of lower levels of SERCA2, could lead to decrease in the apoptotic rates (Rizzuto et al. 2003).

In addition, the Ca²⁺ channels and pumps can directly modify the transcription of genes and their products, e.g., the proteolytically cleaved 75 kDa C-terminal fragment of CaV1.2, an L-type voltage gated Ca²⁺ channel termed calcium channel associated transcriptional regulator or CCAT translocates to the nucleus and alters the transcription of several genes such as *Myc*, Bcl-associated death promoter (*Bad*) and artemin (Gomez-Ospina et al. 2006). Nuclear CCAT protein levels increase or decrease in response to low and high intracellular Ca²⁺, respectively (Chung and Jan 2006; Gomez-Ospina et al. 2006). The isoform-specific upregulation of the mRNA and protein of the plasma membrane Ca²⁺ pump, PMCA4 leads to differentiation of a human colon cancer cell line (Aung et al. 2007). In colon cancer, SERCA3 protein expression is either reduced or completely silenced compared with normal tissue resulting in a loss of differentiation in tumor cells (Gelebart et al., 2002). Similarly, as a result of decrease in SERCA2b expression, androgen-sensitive prostate cancer epithelial cells become androgen insensitive as a result of neuroendocrine differentiation process (Vanoverbergh et al. 2004).

Therapeutic targeting of Ca²⁺ signaling in cancer

In normal cells, the Ca²⁺ signaling is highly regulated spatially such as between ER and mitochondria, between PMCA and ER, including the spatial role played by the cytoskeletal elements and temporally in terms of Ca²⁺ oscillations and Ca²⁺ wave frequencies, amplitudes, and durations (Berridge et al. 2003; Rizzuto and Pozzan 2006; Berridge 1997). However, in cancer cells, such a spatio-temporal regulation of Ca²⁺ signaling is significantly modulated in terms of frequencies, amplitudes and duration of Ca²⁺ signals. Therefore, specific targeting of Ca²⁺ channels or pumps with restricted tissue distribution, altered expression in cancer and/or a role in the regulation of tumorigenic pathways, could specifically disrupt Ca²⁺ homeostasis in cancer cells. Treating both normal and cancer cells with an agent that disrupts these pathways may kill the cancer cell, owing to the loss of redundancy (Ding et al. 2010). One of the approaches in this direction involves selectively altering the activity of the Ca²⁺ channel or pump so as to inhibit Ca²⁺-dependent tumorigenic pathways such as in cell proliferation. It is also possible to exploit the altered expression of the Ca²⁺ channel or pump in cancer cells such as by using an activator of a PMCA that is overexpressed in a cancer cell, Ca²⁺ influx into the cell can then be increased that can lead to activation of cell death pathways and/or disruption of cell-cycle progression. Such a strategy has been adopted in the control of LNCaP prostate cancer cells by use of menthol as an activator of TRPM8 (Mahieu et al. 2007). In addition, by using inhibitors of pumps that either sequester Ca²⁺ into intracellular stores or increase Ca²⁺ efflux across the plasma membrane can lead to excessive [Ca²⁺]_i as well as excessive mitochondrial Ca²⁺ uptake resulting in cell death. An improved knowledge of the functioning of Ca²⁺ channels,

pumps, and exchangers will help in treating cancer (Berridge 1997, 2003; Rizzuto and Pozzan 2006; Wang et al. 2010; Zhong et al. 2010).

Conclusions

The intracellular Ca^{2+} waves generated as a result of concerted activities of several cellular Ca^{2+} channels and pumps provides for a very effective and fast signaling that is needed at every step of the way in the day-to-day life of a living organism. The living cells have evolved to encode these Ca^{2+} waves as *calcium code* for information transfer in terms of the amplitude, duration, frequency, waveform or timing of Ca^{2+} oscillations that are then decoded at a later stage of signal transduction. In cancer cells, the altered expression of these Ca^{2+} channels and pumps cause alterations in Ca^{2+} wave characteristics resulting in a modified *calcium code*. Therefore, one can target these Ca^{2+} channels and pumps as therapeutic options to decrease cancer cell proliferation and increase cancer cell apoptosis. Such novel and highly innovative strategies can provide rationale and approaches for the design and development of novel technologies based on Ca^{2+} waves for the diagnosis and treatment of cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Ca^{2+} signaling in cells. In resting cells the intracellular Ca^{2+} concentration, $[\text{Ca}^{2+}]_i$, is maintained at ~ 100 nM by Ca^{2+} removal via plasma membrane Ca^{2+} -ATPase (PMCA) and Ca^{2+} uptake into ER by SR Ca^{2+} -ATPase (SERCA) transporters. The Na/Ca exchanger (NCX), a major secondary regulator of $[\text{Ca}^{2+}]_i$, is electrogenic, exchanging three Na ions for one Ca^{2+} . Intracellular Ca^{2+} hyperpolarizes many cells by activating K^+ channels, and in some cells, Cl^- channels. This decreases CaV channel activity but increases the driving force across active Ca^{2+} -permeant channels. In excitatory Ca^{2+} signaling, plasma membrane ion channels are triggered to open by changes in voltage, or extra- or intracellular ligand binding. When open, ~ 1 million Ca^{2+} ions/s/channel flow down the 20000 fold Ca^{2+} gradient ($E_{\text{Ca}} \sim +150$ mV). Initial increase in $[\text{Ca}^{2+}]_i$ triggers more release, primarily from ER via Ca^{2+} -sensitive RyR. GPCR or receptor tyrosine kinase-mediated activation of PLC cleaves PIP₂ into IP₃ and DAG. IP₃ is a ligand for the intracellular IP₃R channel spanning the membrane of the ER. GPCRs catalyze the exchange of GDP for GTP on $G\alpha$ subunits, releasing active $G\alpha$ and $G\beta\gamma$ subunits that in turn activate PLC β .

FIG. 2.

Elementary and global events of calcium signaling by IP_3 in a dose-dependent manner. Inositol trisphosphate receptors (IP_3R) are shown arranged in clusters that form Ca^{2+} release sites in the ER. **A:** at low IP_3 concentration, a small number of IP_3R (shown in green) release Ca^{2+} (shown in blue) into the cytoplasm as elementary event called “blip” whereas others IP_3R (shown in dark red) are not bound with IP_3 and therefore are not active. **B:** at intermediate concentration of IP_3 , a group of IP_3R opens to release Ca^{2+} to form “puff” which remains local because the neighboring IP_3R s are not active. **C:** at higher concentrations of IP_3 , the activation of a large number of IP_3R leads to formation and global propagation of Ca^{2+} waves. Ca^{2+} released at one cluster can trigger Ca^{2+} release at adjacent clusters by calcium-induced calcium release (CICR) that leads to the formation of Ca^{2+} waves which propagate by successive cycles of Ca^{2+} release, diffusion, and CICR.

Fig. 3.

Regulation of cell cycle by Ca^{2+} . Ca^{2+} signaling at various key stages of the cell cycle plays crucial roles such as the activation and expression of transcription factors FOS and JUN, CREB) and NFAT as the cells enter the G1 phase. These transcription factors in turn regulate the D-type cyclins, required for activation of cyclin D-CDK4 complexes (D-K4). Later in G1 phase of cell cycle, Ca^{2+} plays an important role in the activity of D-K4 and E-K2 complexes required for phosphorylation and consequently inactivation of retinoblastoma (RB) that causes cells to progress to S phase. During the G₁/S and G₂/M transitory phases of cell cycle, the Ca^{2+} oscillations play important roles in centrosome duplication, maturation, and separation in cytokinesis.

Fig. 4.

Ca²⁺ waves activate transcription factor NFAT that in turn controls cell cycle. The changes in Ca²⁺ waves oscillation frequency can activate transcription factors such as transcription factor NFAT resulting in regulation of cellular transcription. Calcineurin plays a major role in the cell cycle progression through G₁ and S phases by regulating CREB1 and the nuclear NFAT. In its inactive phosphorylated state, NFATs localizes in the cytoplasm, but following an increase in [Ca²⁺]_i, the activated calcineurin dephosphorylates NFAT which then translocates to the nucleus and regulates expression of its target genes. The influx of Ca²⁺ through plasma membrane channels such as the store-operated channel ORAI1 as well as efflux of Ca²⁺ from the ER can activate calcineurin and NFAT signaling pathway for inducing changes in cell cycle gene expression. Experiments using the “calcium clamp” have shown that the sensitivity of different transcription factors to [Ca²⁺]_i oscillations is highly frequency dependent.

Table 1

The changes in the expression of Ca^{2+} channels and pumps in cancer.

Ca^{2+} Channel/pump	Type of Cancer	mRNA level	Protein level	Reference
A. SERCA Pumps				
1. SERCA2	Colon and Lung cancer	↓	ND	Korosec et al. 2006
	Thyroid cancer	↓	↓	Pacifico et al., 2003
	Oral cancer; squamous cell carcinoma	↓	↓	Endo et al., 2004
	Colorectal cancer	↑	ND	Chung et al., 2006
2. SERCA3	Colon cancer	ND	↓	Brouland et al., 2005
B. PMCA Pumps				
1. PMCA	Skin and lung cancer	ND	↓	Reisner et al., 1997
2. PMCA1	Oral cancer; squamous cell carcinoma	↓	↓	Saito et al., 2006
	Breast cancer	↑	ND	Lee et al., 2002
	Skin cancer	↓	ND	Reisner et al., 1997
3. PMCA2	Breast cancer	↑	ND	Lee et al., 2005
4. PMCA4	Skin cancer	↓	ND	Reisner et al., 1997
C. $\text{IP}_3\text{R/RyRs}$				
$\text{IP}_3\text{R2}$	Non-small-cell lung cancer	↑	ND	Heighway et al., 1996
$\text{IP}_3\text{R3}$	Gastric cancer	↑	↑	Sakakura et al., 2003
RyR1	Thyoma	↓	ND	Kusner et al., 1998
D. Voltage-gated Channels				
$\text{Ca}_v1.2$	Colon cancer	↑	ND	Wang et al., 2000
$\text{Ca}_v1.1$	Colorectal cancer	↑	ND	Zhang et al., 1997
$\text{Ca}_v3.1$	Glioma	↑	ND	Latour et al., 2004
	Colorectal cancer	↓	ND	Toyota et al., 1999
$\text{Ca}_v3.3$	Colon cancer	↓	ND	Paz et al., 2003
E. TRP Channels				
TRPV1	Bladder cancer	ND	↓	Lazzeri et al., 2005
	Glioma	↑	↑	Contassot et al., 2004
TRPV6	Prostate cancer	↑	ND	Fixemer et al., 2003
	Breast, ovary, thyroid, and colon cancers	↑	ND	Zhuang et al., 2002
TRPM1	Melanoma	↓	ND	Deeds et al., 2000
TRPM8	Prostate cancer	↑	ND	Schmidt et al., 2006
	Colorectal cancer	↑	ND	Tsavler et al., 2001