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Cell Physiology: Unraveling significance of Calcium Signaling in cell proliferation, gene transcription and apoptosis

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ABSTRACT

Various functions in eukaryotic cell from proliferation upto death are performed by calcium signaling. Calcium level is elevated in stimulated cells which interferes cell growth through various pathways. Ca^{2+} sensitive fluorescent indicators like Fura-2 are used to judge the levels of calcium ions. Intracellular and extracellular sources are used by cell to generate signals for normal functioning. Movement of calcium across various barriers is facilitated by various calcium channels. During cell proliferation, synthesis of DNA, regulation of behavior and structural integrity of microtubules is affected by calcium ions which are associated with CDK or cyclin complexes by CAM and CaN. Changes in intracellular levels of calcium result in translocation of transcription factors like NFAT and NF-κB into nucleus. The phosphorylation of NFAT and NF-κB results in their activation. After activation they move into nucleus and combines with specific genes. Calcium signaling also plays an important role in programmed death of cell through apoptosis. The production of death-inducing signaling complex (DISC) due to some mediators may lead to programmed cell death. Additionally, mitochondrial permeability transition (MPT) may lead to cellular death due to calcium overload. BCL-2 proteins direct apoptosis by monitoring the route of $Ca^{2+}$from the ER to the mitochondria.

Keywords: CaM, CaN , CDK, gene transcription

Introduction

Calcium ions (Ca^{2+}) act as secondary messenger to modulate some important eukaryotic cell functions like growth, proliferation, apoptosis, differentiation and gene transcription. A change in intracellular concentration of calcium ions triggers cellular response. The concentration of Ca^{2+} in cytosol is maintained at 100 nM in unstimulated cells, which is often referred to as basal or resting Ca^{2+} concentration. The levels of Ca^{2+} commonly extend from 0.5 µM to 1 µM. But Ca^{2+} signals more than 1µM are also recorded which may be due to cellular damage and often causes disastrous cellular effects like excito-toxic stimulation of neurons and demise of arterial smooth myocytes which are displayed to calcium aggregates in nature. Some extrinsic factors like hormones, neurotransmitter, growth factors, antibody, temperature and pH change, microbial invasion and some intrinsic cellular cues like spontaneous Ca^{2+}signals within cardiac myocytes and developing neurons could be a cause of occurrence of Ca^{2+}signaling. Stimulation of cells leads to an increase in Ca^{2+} concentration while at end of stimulation, Ca^{2+} concentration returns back to resting state. The Concentration of Ca^{2+}, acquired by a cell is decided by nature of stimulus, factors like severity and interval of stimuli, presence or absence of Ca^{2+} modulating proteins and ATP generating mitochondria the concentration of Ca^{2+} is attained in cells [1]. The levels of Ca^{2+} in cytosol can be judged by using fluorescent indicators which are Ca^{2+} sensitive like Fura-2 by measuring the average across the cell. $Ca^{2+}$is involved in various pathologies and natural aging processes. Some signaling functions in cell may be performed by basal Ca^{2+} levels because increase in Ca^{2+} concentration can stop some processes within cell. For instance, at resting Ca^{2+} concentration a protein named downstream regulatory element antagonist modulator (DREAM) acts as transcriptional repressor. DREAM performs its function by attaching with target genes and prevents their transcription[2]. The lack of Ca^{2+} concentration may activate self-eating of cell (removal of unwanted organelle and proteins due to lysosomes) or quiescence (Proliferation arrest). Cells may utilize intracellular or extracellular sources of calcium ions to generate signals. This utilization of intracellular and intracellular sources can vary. Inositol 1,4,5-trisphosphate receptors (IP3Rs), the mucolipin subfamily of transient receptor potential channels, two-pore channels (TPCs)[3], ryanodine receptors (RyRs) and VGCC are some principal Ca^{2+} releasing channels. Sarcoplasmic reticulum (in muscle cells) and endoplasmic reticulum (in non-excitable cells and neurons) act as major intracellular Ca^{2+} sources. Organelles like the Golgi, nuclear envelope, lysosomes, and other acidic vesicles also take part in Ca^{2+} signaling in stimulated cells.

Role of calcium signaling in cell physiology

Calcium signaling plays a vital role in physiology of different body cells to perform their functions. Some of them are quoted below

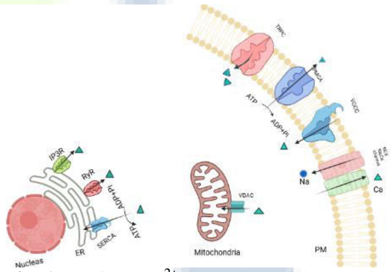

Fig. 1: Various Ca^{2+}channels are illustrated.

In cellular proliferation

Ca^{2+} signals play a key role in regulation of cell cycle progression. During conversion from G1 to S phase, the G2 to M phase, and the metaphase to anaphase the concentration of Ca^{2+} inside the cell rises. During early G1 phase (when cell again enters cell cycle) Ca^{2+} is involved in the activation of the nuclear factor of activated T-cell (NFAT), AP1 (FOS and JUN) transcription factors and c-AMP-responsive element binding protein. Expression of protein complexes focused on cyclin-dependent protein kinases (CDKs) is the primary administrator of the cell cycle, the entry of cell into the subsequent phase of the cell cycle is regulated by it when it binds to a cyclin. Ca^{2+} ions and CDK/cycline complexes are linked by Ca^{2+}-sensors calmodulin (CAM) and calcineurin (CaN). Intermediary proteins like CAMKI, CAMKII, and CAMKIII together with CAM and CaN, interplay with CDK/cycline complexes to mediate important events during cell cycle such as synthesis of DNA through CAMKI regulated cyclin D1-CDK4 and regulation of behavior and structural integrity of microtubules by reducing the amount of Ca^{2+} necessary for depolymerization of microtubule and by interplaying with nucleoporin p62 [4] and for accomplishment of cytokinesis. Exposure to W-7 and W-13 CAM antagonists may lead to downregulation of cyclins and amplification of p21 resulting arrest at G1 phase of cell cycle. The synthesis of DNA is reduced in a dose-dependent manner when injections of monoclonal antibodies are used against CAM. It is worthwhile describing a few instances where alterations in Ca^{2+} signaling can result in disruption in cell cycle and subsequent effects on tumor pathology. Guanosine exchange factors, which are RAS stimulators and GTPase activating protein, which is RAS inhibitors, are supervised by cytosolic Ca^{2+} levels. Upon cytoplasmic cyclin D1 overexpression, RAS phosphorylates the tumor suppressor RB1, triggering the cell's transformation into the S phase. This, in turn, activates the proliferative mitogen-activated protein kinase (MAPK) pathway. The G1/S shifting checkpoint can be removed by cancer cells with constitutively elevated cytosolic Ca^{2+} levels, which can result in unchecked proliferation. Cyclin D and cyclin E overexpression play chief role in the dysregulation of the cell cycle in breast cancer; in fact, overexpression of the cyclin D1 and E proteins is seen in over 50% of breast cancers. VGCCs (Voltage gated

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calcium channels) are also linked to the control of cell growth. Certain genes belonging to the VGCCs family are downregulated resulting brain, kidney, breast, and lung cancers, revealing that these Ca^{2+} channels function as tumor suppressors. Members of the VGCCs family are expressed at detectable levels in melanoma cells but are not expressed untransformed melanocytes [5].

In gene transcription

Proteins like calmodulin (CaM), calmodulin-dependent protein kinase II (CaMKII), and calcineurin are crucial for cell cycle progression and are part of the Ca^{2+} signaling pathways. The activation and differential expressions of genes related to these proteins are involved in cellular networks. The expression of CaM increases during the shifting from the G1 phase to the S phase of the cell cycle. Modulating CaM levels modifies proliferation by interfering with DNA replication and disrupting G1 and M-phase progression. Levels of Ca^{2+} outside the cell, activity and expression of CaM greatly affect the machinery of cell cycle. The interaction between tumor suppressor proteins Rb and E2F, a transcription factor required for progression of cell cycle, and is often dephosphorylated, may take place. Cdk4 and Cdk2 (cyclin-dependent kinases) may phosphorylate Rb which may result in ceastion of Rb-E2F interaction, which also induces advancement towards the G1-S checkpoint. CaMKII disrupts the G2-M phase and advances the G1-S phase, which is consistent with the effects of CaM on cell cycle suppression. Calcium (Ca^{2+}) signaling is crucial during the cell cycle, particularly for breaking down the nuclear envelope. These cellular processes are regulated by gene networks involved in various pathways. Changes in intracellular Ca^{2+} levels cause transcription factors like NF-κB and NFAT to move from the cytosol into the nucleus, where they play important roles. In resting cells, NF-κB is kept in the cytoplasm due to presence of its inhibitor (IκB). This anchor of NF-κB covers up nuclear localization signals (NLS) that would normally tell NF-κB to move into the nucleus. Brief fluctuations in Ca^{2+} trigger a series of phosphorylation events that ultimately lead to the activation of NF-κB. IκB kinase (IKK2) inhibits IκB via phosphorylation, allowing IκB to separate from NF-κB and reveal its nuclear localization signals. PKC or Ca^{2+}/CaM dependent protein kinase (CaMK, type I, II, or III) [6] can both activate IKK2. Various inflammatory pathways are linked to transcription of gene networks, such as cell proliferation, which is activated by the transfer of NF-κB in the nucleus [6].

Immune response, development of organs and system-like heart, muscles and CNS and regulation of cell cycle is interfered by NFAT. Regarding NF-κB, NFAT is located in the cytoplasm of the resting cell, where it is dormant and has phosphorylation at these sites covering its NLS. Calcineurin (CaN), an enzyme that is dependent on Ca^{2+} and CaM, dephosphorylates NFAT during cell activation, exposing NLS. As a result, NFAT binds to target genes' cis-regulatory components by moving in the nucleus. CaN's translocation into the nucleus is regulated to maintain the proper ratio of activated to non-activated CaN. Because Ca^{2+} has a greater affinity for CaN, small Ca^{2+} transients in cytoplasm are adequate to activate NFAT; nevertheless, in many cases, signal amplification of Ca^{2+} release is induced by SOCE is also necessary for activation of NFAT in various cell kinds. On the other hand, large Ca^{2+} transient amplitudes are required for NF-κB activation. Furthermore, prolonged alterations following NFAT activation are thought to be less effective for some T-cells, stem cells, and dendritic cells than Ca^{2+}oscillations [7].

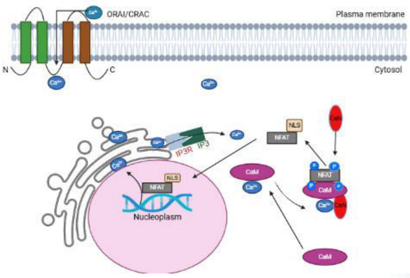

Fig. 2: Ca^{2+} signaling controls NFAT and the activity of transcription factors.

In apoptosis

Evading programmed cell death (PCD) is a foremost characteristic of tumor cells. Decline in the functionality of cells can trigger various intracellular pathways ultimately leading to death of cell. Apoptosis is a well-known type of PCD. Apoptosis is a tightly governed process that usually shows up as membrane bulging, mitochondrial malfunctioning, DNA and nuclear disintegration, chromatin compression, and even when the cell membrane is undamaged. The link between cell-impermeant inputs and cell membrane stimulates membrane receptors, which in turn activates the so-called intrinsic pathways. Therefore, extracellular ligands like tumor necrosis factor-α (TNFα) and first apoptosis signal (FAS) ligand, as well as their receptors, TNFRs and FAS respectively, are necessary for membrane-induced apoptosis. These activated receptors use many mediators, causes the production of death-inducing signaling complex (DISC) as some adaptors may carry death effector domain. DISC activates initiator caspase-8's autoproteolytic cleavage, which activates caspase-3, -6, and -7, the executioner caspase. The primary morphological characteristics of apoptosis rely on this proteolytic cascade because it dramatically enhances first signal and starts splitting of many cellular targets. Apoptosis also involves the initiation of an intracellular, mitochondria-centered pathway. This process links irreversible mitochondrial stress to cellular death. However, a variety of stressors can result the mitochondrial permeability transition (MPT), including as hypoxia, changes to ETC, unprotected generation of ROS, and asymmetrical homeostasis of mitochondrial protein [7,8]. MPT causes an osmotic imbalance in the mitochondria and permeabilizes the outer membrane of the mitochondria, which discharges various mitochondrial proteins, including cytochrome C (cyt-C). Specifically, a multiprotein complex is formed between cytochrome-C and APA (apoptotic protease activating) factor 1 that can engage and initiate the initiator caspase-9 through the caspase recruitment domain. Afterwards, caspase-9 cleaves and activates caspase-3, -6, and -7, which are the other executioners. The mitochondrial Ca^{2+} load is a primary controlling mechanism of the intrinsic route. Krebs cycle dehydrogenases are markedly regulated by Ca^{2+}, which is typically settled in the matrix of mitochondria at higher concentrations. Ca^{2+}overload under certain circumstances can result Mitochondrial Permeability Transition Pore by permitting the pore to open, which then causes the emission of apoptogenic components. The key mechanism controlling both apoptosis and mitochondrial Ca^{2+}remodeling is Ca^{2+}release from the ER. B-cell lymphoma-2 (BCL-2) proteins are categorized into two groups: pro-apoptotic (such as Bax, Bak, Bim, Bid, etc.) and antiapoptotic (such as BCL-2, BCL-xL, and Mcl-1). Research shows that BCL-2 proteins with anti-apoptotic attributes direct the apoptotic program by monitoring the route of $Ca^{2+}$from the ER to the mitochondria in both organelles. Recent research has revealed that these proteins have antiapoptotic attributes at the level of Mitochondria-associated endoplasmic reticulum membranes (MAMs). It was shown that overexpression of pro-apoptotic BCL-2 proteins decreases both ER- Ca^{2+} release through either reducing the Ca^{2+}concentration of the ER or directly controlling IP3R3-induced pore opening. Consequently, the apoptotic program is abolished and Ca^{2+}-induced MPT is inhibited. Moreover, it has been shown that Ca^{2+}oscillations mediated by IP3Rs, which are necessary to boost production of mitochondrial energy and encourage cellular proliferation, are determined by BCL-2, BCL-XL, and Mcl-1. All together, these proteins affect three critical facets of the development of cancer: energy production, survival, and cell death. Pro-apoptotic BCL-2 members were consistently upregulated in human cancer samples and linked to colon, breast, and stomach cancer invasion and metastasis. Not only BCL-2 proteins but some other proteins also modulate the release of ER- Ca^{2+} into mitochondria to control apoptosis and cell growth. The creation of tumors and the upkeep of the tumor environment are crucial functions performed by oncogene RAS. RAS deregulates ER Ca^{2+} dynamics to carry out this function, which inhibits apoptosis, impairs mitochondrial metabolism, and increases the survival of malignant cells. Furthermore, a number of proteins that are often associated in promoting the Ca^{2+}-dependent apoptotic response including Bad, Bax, and hexokinase-2 are phosphorylated and delivered inactive by the oncogene AKT. Additionally, by directly controlling IP3R3 opening, AKT hinders the Ca^{2+}overload required to stimulate intrinsic apoptosis, hence inhibiting the apoptotic process.

Conclusion

Calcium signaling plays an integral role in the physiology of individuals. Disruptions in calcium signaling may lead to abnormal division of cells. Calcium signaling interprets various pathways to control eukaryotic cell functions. By interacting with various proteins, calcium signaling influences gene transcription. Research on calcium signaling may lead us to understand the division of cells and control of abnormal cell division.

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