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# Calcineurin in cancer signaling networks

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## ABSTRACT

Calcium/calcineurin/nuclear factor of activated T-cell signaling is a vital regulator of the development and function of immune, nervous, cardiovascular, and musculoskeletal systems. The dysregulation of calcineurin activity has been implicated in various pathological conditions, including certain cancers, cardiac hypertrophy, and neurodegenerative disorders. Calcineurin is highly expressed in certain cancers and stabilizes and activates factors that promote cancer cell proliferation. Research has shown that protein dephosphorylation by calcineurin contributes to tumor formation and progression. Thus, elucidating molecular mechanisms of calcineurin-mediated tumorigenesis and tumor cell growth and targeting signaling pathways downstream of calcineurin may lead to new cancer therapies. This study reviewed the multiple roles of calcineurin in cell cycle progression and its potential as a target for cancer treatment.

Keywords: calcineurin, calcium, dephosphorylation, prognosis in cancer, cell proliferation

Abbreviations:
CnA: calcineurin A
CnB: calcineurin B
ERα: estrogen receptor-α
NFAT: nuclear factor of activated T cell



## INTRODUCTION

The serine/threonine phosphatase family includes PP1, PP2A, calcium ($Ca^{2+}$)-dependent PP2B (calcineurin), magnesium-dependent PP2C, and PP2A-like phosphatases, such as PP4 (PPX), PP6, PP5, and PP7.[1] Calcineurin is a $Ca^{2+}$-dependent phosphatase activated by elevated intracellular $Ca^{2+}$ levels and plays critical roles in numerous cellular processes, making it an important regulator of various physiological functions and a significant target for therapeutic interventions. Calcineurin was originally discovered as an inhibitor of $Ca^{2+}$- and calmodulin-dependent phosphodiesterase.[2] Calcineurin is the target of immunosuppressant drugs, such as cyclosporin A and FK506, forming complexes with intracellular binding proteins to inhibit calcineurin activity. This mechanism is the basis for preventing organ transplant rejection and treating autoimmune diseases. Intracellular

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$Ca^{2+}$ initiates various physiological reactions and regulates biological processes, including cell proliferation and death, differentiation, muscle contraction, and neurotransmission.[3,4] The exact $Ca^{2+}$ concentration depends on the cell type and condition. However, generally, it is approximately several micromolars outside the cell, whereas it ranges from 10 to 100 nM inside the cell. This significant difference in concentration is crucial for signal transduction through $Ca^{2+}$ influx and efflux. Even within the cell, the $Ca^{2+}$ concentration varies by organelle: ~100 nM in the cytosol, 10–100 nM in the nucleus, 0.5–1 mM in the endoplasmic reticulum (ER), and ~100 nM in the mitochondria.[5] Intracellular $Ca^{2+}$ levels are tightly regulated by $Ca^{2+}$ channels and pumps in the plasma membrane, ER, and mitochondria. Several channels, pumps, and $Ca^{2+}$ regulatory proteins are highly expressed in cancer. Abnormal $Ca^{2+}$ signaling and altered $Ca^{2+}$ concentrations are linked to tumor growth and resistance to anticancer therapy.[3,4] Therefore, understanding $Ca^{2+}$ homeostasis abnormalities in cancers and effectively controlling intracellular $Ca^{2+}$ levels are crucial for developing new anticancer drugs. Comprehensive gene expression and proteomic analyses have revealed that intracellular $Ca^{2+}$ regulates gene expression, which is important for cancer cell proliferation. This review introduced these molecular mechanisms, highlighted the importance of the calcineurin/nuclear factor of activated T-cell (NFAT) pathway in cancer, and provided perspectives on the effectiveness of cancer therapies that target $Ca^{2+}$ signaling.

## STRUCTURE AND ISOFORM OF CALCINEURIN

Calcineurin is a heterodimeric protein consisting of a catalytic subunit (calcineurin A [CnA]) and a regulatory subunit (calcineurin B [CnB]). Mammalian CnA has three isoforms (CnAα, CnAβ, and CnAγ) and is encoded by *PPP3CA*, *PPP3CB*, and *PPP3CC*, respectively (Fig. 1). CnAα and CnAβ share 81% similarity, but the main difference is the unique proline repeat sequence at the N terminus of CnAβ, which has an important role in substrate recognition. CnAβ has two splice variants (CnAβ1 and CnAβ2). The CnAβ1 isoform is ubiquitously expressed and considered the canonical form of the CnAβ subunit. The main differences between the isoforms are their expression patterns and functional roles. CnAγ is specific to the testis and brain, whereas the other is expressed ubiquitously, especially in the brain. CnA consists of an

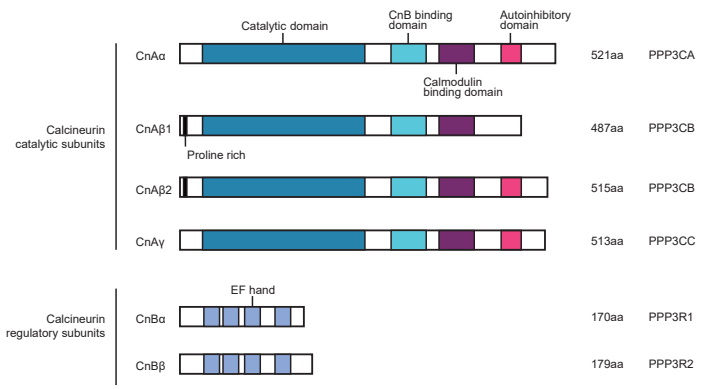


**Fig. 1** Schematic illustration of the structure of CnA and CnB subunits
In mammals, three genes encode CnA (CnAα, CnAβ, and CnAγ), and CnAβ contains two splice variants (CnAβ1 and CnAβ2).
CnA: calcineurin A
CnB: calcineurin B

N-terminal catalytic domain, a domain that binds to CnB and calmodulin, and a C-terminal autoinhibitory domain.

The regulatory subunits of calcineurin (CnBα and CnBβ, with 83% sequence homology) are encoded by *PPP3R1* and *PPP3R2* genes, respectively. CnBα is expressed in all tissues, whereas CnBβ expression is limited to the testis. CnB has four EF-hand motifs for $Ca^{2+}$ binding and is structurally similar to calmodulin.

## REGULATORY MECHANISM OF CALCINEURIN ACTIVITY

Increased intracellular $Ca^{2+}$ levels induce $Ca^{2+}$ binding to CnB and calmodulin, leading to an interaction between calmodulin and calcineurin. This interaction displaces the autoinhibitory domain of CnA from its active site, activating calcineurin and subsequently dephosphorylating its target protein. Calcineurin activity is regulated by multiple regulators (Fig. 2). Calcineurin is primarily localized in the cytoplasm, but a small amount of calcineurin also functions in the nucleus.[6] CnA is activated by intramolecular cleavage by two proteases (calpain and caspase-3).[7-9] Following elevated intracellular $Ca^{2+}$ levels, calpain, a $Ca^{2+}$-dependent cysteine protease, can cleave calcineurin, which removes its autoinhibitory domain, leading to its activation. Conversely, several mechanisms can inactivate calcineurin.

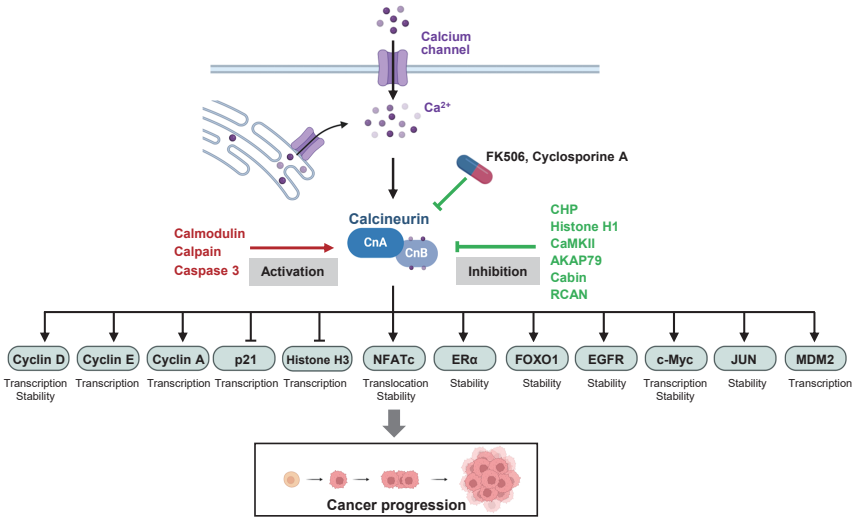


**Fig. 2** Calcineurin signaling pathway to activate cancer cell proliferation
Increased cytosolic $Ca^{2+}$ concentrations, for example, by activating $Ca^{2+}$ channels, activate calmodulin, which in turn activates calcineurin composed of CnA and CnB. Activated calcineurin dephosphorylates and regulates NFAT transcription factors and other targets. Cyclosporine and FK506 are calcineurin inhibitors. Factors important in the regulation of calcineurin activity are shown in the figure. Calcineurin stabilizes, transcriptionally activates, and alters the localization of factors that promote and activate cell proliferation, promoting cancer cell proliferation.
AKAP: A-kinase anchoring protein
CnA: calcineurin A
CnB: calcineurin B
EGFR: epidermal growth factor receptor
ERα: estrogen receptor-α
NFAT: nuclear factor of activated T cell
RCAN: regulator of calcineurin

**Table 1** Calcineurin substrates

| Category | Substrates | Dephosphorylation sites | Reaction by dephosphorylation | Source |
|---|---|---|---|---|
| Transcription factor/ transcriptional regulation | c-Jun | S243 | Stabilizes c-Jun, promotes the interaction between c-Jun and Sp1 | Huang et al,[20] 2008 |
| | c-Myc | T58, S62 | Inhibits the binding to Fbxw7 and promotes stability | Masaki et al,[21] 2023 |
| | DAXX | S669 | Promotes H3.3 uptake by DAXX | Michod et al,[22] 2012 |
| | E2F1 | S403 | Inhibits the binding to Fbxw7 and promotes stability | Sato et al,[23] 2024 |
| | EGFR | S1046, 1047 | Promotes stability | Masaki et al,[24] 2023 |
| | ERα | S294 | Promotes stability and activity | Masaki et al,[25] 2021 |
| | FOXO1 | T24 | Promotes stability | Tomiyasu et al,[26] 2024 |
| | MEF2 | S221, S255, S408 | Activates MEF2 and promotes the change from sumoylation to acetylation of L403 | Flavell et al,[27] 2006 |
| | MLL3 | ND | Activates MLL3 and increases H3K4me1, leading to activation of cardiac hypertrophy regulatory genes | Pane et al,[28] 2024 |
| | NF1 | ND | Promotes activity | Brun et al,[29] 2013 |
| | NFATC1 | S172 | Promotes nuclear transfer and stability of NFATC1 | Chow et al,[30] 2000, Hanaki et al,[31] 2023 |
| | NFATC2* | Five residues among following sites, S170, S173, S174, S176, S177, S179, S182 in SRR-1 domain | Promotes nuclear transfer and stability of NFATC2 | Hanaki et al,[31] 2023, Okamura et al,[32] 2000 |
| | | S215, S219, S223 in SP-2 domain | | |
| | | S270, S276, S278, S282 in SP-3 domain | | |
| | | S328 in KTS motif | | |
| | NFATC4 | S170 | Promotes nuclear transfer of NFATC4 | Kim et al,[33] 2010 |
| | TFEB | S142, S211 | Promotes nuclear transfer of TFEB | Medina et al,[34] 2015 |
| Kinase/ phosphatase/ their related protein | ASK1 | S967 | Promotes the dissociation of ASK1 from the 14-3-3 protein, resulting in the activation of ASK1 | Liu et al,[35] 2006 |
| | CaMKIIγ | S334 | Promotes nuclear transfer | Woolfrey et al,[36] 2015 |
| | DARPP-32 | T34 | Inactivates DARPP-32, leading to activation of PP1 | Greengard et al,[37] 1999 |
| | Inhibitor-1 | ND | Inactivates inhibitor-1, leading to activation of PP1 | Mulkey et al,[38] 1994 |
| | MYPT1 | T696 | Affects actin polymerization by activating MP and improves endothelial barrier function | Kolozsvári et al,[39] 2012 |
| | RIIα | S95 | ND | Blumenthal et al,[40] 1986 |
| | RCAN1 | S108, S112, T124, T192 | ND | Li et al,[41] 2020 |

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| Category | Substrates | Dephosphorylation sites | Reaction by dephosphorylation | Source |
|---|---|---|---|---|
| Cell cycle/apoptosis | BAD | S155 | Promotes heterodimerization of BAD and Bcl-xL, which induces apoptosis | Wang et al,[42] 1999 |
| | Cyclin D1 | T286 | Stabilizes cyclin D1, inducing G1/S progression | Goshima et al,[43] 2019 |
| | DRP1 | S637 | Induces Drp1 translocation to mitochondria, promoting mitochondrial fission and fragmentation | Cereghetti et al,[44] 2008 |
| | Dynamin 1 | S774, S778 | Promotes endocytosis of TrkA receptors and axonal growth | Bodmer et al,[45] 2011 |
| | RB | S795 | Inducing release of the repressor complex and activating calcium-dependent transcription of neuronal genes | Qiu et al,[46] 2008 |
| Cytoskeleton | MAP2 | ND | ND | Goto et al,[47] 1985 |
| | Tau | T181, T231 | ND | Garver et al,[48] 1999 |
| | | S199, S202, S262, S356, S396, S404 | ND | Wei et al,[49] 2002 |
| | Tubulin | ND | ND | Goto et al,[47] 1985 |
| Scaffold protein | AKAP79/150 | ND | Triggers the reorganization and adjustment of signaling pathways | Coghlan et al,[50] 1995 |
| | KSR2 | S198, T287, S310 | Activates ERK, induces membrane localization of KSR2 | Dougherty et al,[51] 2009 |
| Ion channels/ membrane channels/ their related protein | GluA1 | S845 | Promotes removal of AMPARs from synapses and endocytosis | Sanderson et al,[52] 2016 |
| | Kv2.1 | S11, S453, S537, S563, S603, S651, S715 | Induces a hyperpolarizing shift in the voltage-dependent activation of channels, causing a decrease in neuronal excitability | Park et al,[53] 2006 |
| | Kv4.2 | S552 | Enhance surface expression of Kv4.2-AKAP79 complexes, regulating neuronal excitability and modulating synaptic plasticity | Lin et al,[54] 2011 |
| | mGlu5 | ND | Reduces mGluR desensitization | Alagarsamy et al,[55] 2005 |
| | NHE1 | T779 | Inhibits NHE1 activity | Hendus-Altenburger et al,[56] 2019 |
| | NMDA receptor | ND | Shortens the duration of NMDA receptor channel openings | Lieberman et al,[57] 1994 |
| | TRESK | S276 | Increases $K^+$ current, decreases channel responsiveness to calcium signals | Czirják et al,[58] 2004 |

AKAP: A-kinase anchoring protein
EGFR: epidermal growth factor receptor
ERα: estrogen receptor-α
ERK: extracellular signal-regulated kinase
ND: not determine
NFAT: nuclear factor of activated T cell
RCAN: regulator of calcineurin
*The amino acid residues of the protein indicated by asterisks are dephosphorylated when the cells are stimulated with ionomycin. It is not known whether calcineurin directly dephosphorylates them.

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A-kinase anchoring protein (AKAP) inhibits the phosphatase activity of calcineurin by binding to calcineurin.[10] In addition, AKAP anchors calcineurin and protein kinase A in close proximity, which can modulate calcineurin activity by regulating its access to substrates and other regulatory proteins. Calcineurin-binding protein 1 and regulator of calcineurin (RCAN) also directly bind to and inhibit its activity.[11-14] RCAN transcription is activated by calcineurin, acting as a negative feedback regulator to control excessive calcineurin signaling. Calcineurin homologous protein competes with CnB to bind to CnA, inhibiting calcineurin activity.[15] In addition, histone H1 inhibits CaMKII and calcineurin by blocking calmodulin autophosphorylation.[16] Ser197 phosphorylation of calcineurin via CaMKII blocks $Ca^{2+}$/calmodulin binding to calcineurin, inhibiting its activity.[17-19]

Whereas many kinases are regulated by calmodulin, calcineurin is the only phosphatase directly regulated by $Ca^{2+}$/calmodulin. Calcineurin substrates are transcription factors,[20-34] kinase/phosphatase/their related protein,[35-41] proteins involved in cell cycle/apoptosis,[42-46] cytoskeleton,[47-49] scaffold proteins,[50,51] and ion channels/membrane channels/their related protein[52-59] (Table 1). The best characterized substrate of calcineurin is the NFAT transcription factor, as mentioned later.

## CALCINEURIN/NFAT CASCADE IN CELL PROLIFERATION

Calcineurin signaling in T lymphocytes was first defined as a factor that dephosphorylates the NFAT transcription factor and promotes its nuclear translocation, activating NFAT. The human NFAT family consists of five distinct genes: NFAT1 (also known as NFATc2), NFAT2 (NFATc1), NFAT3 (NFATc4), NFAT4 (NFATc3), and NFAT5 (TonEBP). In addition to promoting nuclear translocation, NFAT dephosphorylation by calcineurin prevents its ubiquitin-mediated degradation by Skp2.[31] $Ca^{2+}$/calcineurin/NFAT signaling is involved in cell cycle control and transcriptional regulation.

The role of calcineurin in cardiac hypertrophy has been reported. In cardiac tissues, NFAT3, which is dephosphorylated by calcineurin, interacts with the cardiac zinc finger transcription factor GATA4 and promotes transcription genes involved in the hypertrophic response, resulting in cardiac hypertrophy.[60] A link between calcineurin and cardiac hypertrophy via epigenetic regulation has been recently reported. Calcineurin forms a complex with MLL3, a histone amino-methyltransferase. Under cardiac stress, carabine, one of the inhibitors, dissociates from the calcineurin-MLL3 complex, inhibiting calcineurin phosphatase activity. As a result, MLL3 is dephosphorylated and activated, leading to increased H3K4me1 levels and the expression of cardiac hypertrophy regulatory genes.[28]

Proteomic analysis has revealed that the expression of many proteins is disrupted by the calcineurin inhibitor FK506.[61] Among these, several histones were upregulated by FK506. The proper regulation of histone proteins is important for cell proliferation because histone overexpression significantly inhibits cell proliferation.[62] NFATc1 has been identified as a transcription factor that represses histone H3 transcription, revealing a novel repressive mechanism through which the $Ca^{2+}$/calcineurin/NFAT pathway escapes the detrimental effects of excess histones.[61] In addition, calcineurin suppresses the transcription of p21, a CDK inhibitor,[63] and activates *MDM2*,[64] *CCND* (encoding cyclin D),[65] *CCNE* (encoding cyclin E),[66] and *CCNA* (encoding cyclin A)[66] transcription, enhancing Cdk activity (Fig. 2).

## CALCINEURIN PROMOTES THE STABILITY OF FACTORS INVOLVED IN CANCER CELL PROLIFERATION

In addition to NFAT, calcineurin regulates downstream signaling pathways by dephosphorylating various substrates. Calcineurin blocks the degradation of cyclin D1,[43] estrogen receptor-α (ERα),[25] FOXO1,[26] epidermal growth factor receptor (EGFR),[24] c-Myc,[21] JUN,[20] and E2F1[23] by dephosphorylation (Fig. 2). Cyclin D1 forms a complex with Cdk4 or Cdk6 to phosphorylate and inhibit RB, promoting $G_1$/S-phase progression. Cyclin D1 is overexpressed in many cancers, including breast cancer, and cyclin D1 overexpression correlates with cancer aggressiveness and metastasis.[67] The main reason for cyclin D1 overexpression in cancer is aberrant proteolysis. Cyclin D1 is ubiquitinated and degraded by Skp/Cullin/F-box-containing E3 ubiquitin ligase upon Thr286 phosphorylation. Calcineurin inhibits cyclin D1 degradation by dephosphorylating Thr286 in cyclin D1, promoting $G_1$/S-phase progression.[27]

A correlation between calcineurin expression and the prognosis of cancer patients has been demonstrated. In ER-positive breast cancer, high calcineurin expression is associated with a higher breast cancer recurrence rate after endocrine therapy and a poorer prognosis.[28] Calcineurin enhances ERα function by (1) dephosphorylating ERα, inhibiting ubiquitin ligase binding and preventing ERα degradation, and (2) promoting ER activity via mammalian target of rapamycin kinase.

Therefore, calcineurin promotes cancer cell proliferation. $Ca^{2+}$ also mediates FOXO1 stability.[26] FOXO1 mutations are concentrated around Thr24, a key amino acid phosphorylated by AKT.[68] These mutations are likely to disrupt Thr24 phosphorylation, leading to FOXO1 dephosphorylation, activation, and stabilization in cancer cells. Indeed, calcineurin dephosphorylates Thr24, leading to FOXO1 stabilization, which promotes cancer cell proliferation by activating *MDM2* transcription and subsequently p53 degradation.[26] In addition, calcineurin directly dephosphorylates and stabilizes proteins, such as c-Myc, EGFR, and E2F1, which promote cancer cell proliferation. Thus, calcineurin is involved in cancer cell proliferation through multiple pathways.

## DYSREGULATION OF CALCINEURIN IN CANCER

In cancer, calcineurin is activated via cleavage by proteases, mutations within the autoinhibitory domain, and transcriptional activation. As mentioned previously, calcineurin has an autoinhibitory domain at its C terminus that negatively regulates phosphatase activity. CnA is cleaved and activated in cancer cells[29] and Alzheimer's disease brains.[69] Missense mutations in Pro484 of CnAα in this autoinhibitory domain are frequent somatic mutations in cancer.[21] Indeed, the Pro484 mutation increases the stability and transcriptional regulatory activity of c-Myc. CnAα is mutated and activated within the autoinhibitory domain in several cancers.

Three genes encoding CnA (*PPP3CA*,[25,70,71] *PPP3CB*,[29,71-73] and *PPP3CC*[74]) are differentially associated with cancer (Table 2). High *PPP3CA* expression is associated with poor prognosis in some cancers, such as ERα-positive breast cancer patients treated with endocrine therapy.[25] CnAα also plays an important role in neurodegenerative diseases and is associated with the abnormal phosphorylation of tau protein, a hallmark of neurodegenerative diseases such as Alzheimer's disease and chronic traumatic encephalopathy.[75,76] In contrast, high *PPP3CB* expression is associated with good prognosis in pancreatic cancer and high-grade gliomas (glioblastoma).[73,77] *PPP3CB* upregulation could affect glioma cell proliferation and apoptosis. *PPP3CB* expression is strongly associated with immune cell infiltration and immune checkpoint genes, which profoundly affect tumor immunity. In contrast, the cleaved and activated forms of CnAβ have been observed in

**Table 2** Correlation between expression level and cancer prognosis

| Genes | Indications of expression and association with cancer | Source |
|---|---|---|
| *PPP3CA* | Associated with a poor prognosis in ovarian cancer | Xin et al,[70] 2019 |
| | Associated with a poor prognosis in pancreatic cancer | Hang et al,[71] 2021 |
| | Associated with a poor prognosis in breast cancer | Masaki et al,[25] 2021 |
| *PPP3CB* | Associated with a poor prognosis in neuroblastoma | Shakhova et al,[72] 2019 |
| | Associated with a good prognosis in pancreatic cancer | Hang et al,[71] 2021 |
| | Associated with a good prognosis in high-grade gliomas | Li et al,[73] 2024 |
| | CNAβ is cleaved and activated in malignant gliomas cells | Brun et al,[29] 2013 |
| *PPP3CC* | Associated with a good prognosis in lung adenocarcinoma | Zhao et al,[74] 2024 |

Associations between the expression of the three genes encoding CnA and cancer prognosis are shown here.

These findings highlight that the role of each gene varies with the type of cancer and that its impact can be tumor-promoting or tumor-suppressive, depending on the situation.

CnA: calcineurin A

malignant glioma cells.[29] *PPP3CC* is a potential tumor suppressor gene in various cancer types, and its decreased expression is linked to cancer progression and poor prognosis. *PPP3CC* knockdown increases cell proliferation and decreases apoptosis in ovarian cancer and glioma cells.[78,79]

Collectively, these studies have indicated that *PPP3CA*, *PPP3CB*, and *PPP3CC* act differently in different cancers, and their roles in cancer are context-dependent. Each of the three CnA isoforms has substrate specificity.[80] Furthermore, there may be nonspecific functions of enzyme activity. Several transcription factors bind to the promoters of calcineurin catalytic and regulatory genes, but little is known about their transcriptional regulation, and the contribution of these factors remains unknown. Other than the regulation mentioned above, changes in gut microbiota composition activate Toll-like receptor signaling, leading to $Ca^{2+}$ influx and calcineurin activation.[81] Thus, calcineurin controls microbiota-dependent intestinal tumor development.

Among NFAT family members, NFATc1 plays multiple roles in cancer cell regulation. NFATc1 promotes the development of various cancers, including prostate,[82] colorectal,[83] and pancreatic[84] cancers. However, it also functions as a tumor suppressor.[85] The calcineurin/NFAT pathway is often abnormally activated in several cancers, and calcineurin and NFATc1 depletion can inhibit cell cycle progression in various cancer cells.[43,84]

Several studies have examined calcineurin inhibitors, such as cyclosporine A and FK506, as potential anticancer agents. Cyclosporine A potentiates the anticancer effect of crizotinib in non-small cell lung cancer xenograft models by inhibiting extracellular signal-regulated kinase (ERK) 1/2 activation, suggesting that inhibiting $Ca^{2+}$/calcineurin/ERK signaling is a promising strategy to enhance the clinical efficacy of crizotinib.[86] Cyclosporine A and FK506 induce apoptosis in lymphoma and leukemia cell lines and increase the survival of T-cell acute lymphoblastic leukemia in mouse models.[87] In addition, these treatments decrease the proliferation and migration of bladder[88] and prostate[82] tumors. FK506 is primarily used as an immunosuppressant to prevent organ transplant rejection. Its immunosuppressive properties could potentially increase the risk of infections and other complications, owing to a weakened immune system. FK506 significantly suppresses tumorigenesis without affecting the liver, kidney, lung function or the tumor immune microenvironment. FK506 induces $G_1$/S-phase arrest in oral squamous cell carcinoma (OSCC) by downregulating cyclin D1, cyclin E1, and c-Myc expression. These findings suggest that FK506

is a safe therapeutic agent for treating OSCC.[77] However, their use is associated with side effects, such as hypertension, nephrotoxicity, and neurotoxicity. In the future, it is important to conduct further studies to investigate the newly discovered molecular mechanisms of calcineurin activity and regulation so that signaling pathways downstream of calcineurin activation can be targeted more specifically.

## TARGETING CALCINEURIN ACTIVATION FOR CANCER TREATMENT

The regulation of $Ca^{2+}$ concentrations in cellular compartments relies on $Ca^{2+}$ channels, pumps, transporters, and $Ca^{2+}$-buffering proteins. Among these, $Ca^{2+}$ channel inhibitors exhibit tumor-suppressive effects in various cancer types. In breast cancer cells, the $Ca^{2+}$ channel blocker amlodipine inhibits cell proliferation, induces apoptosis, suppresses colony formation, and reduces the metastatic potential. When A549 lung cancer cells were treated with amlodipine, there was decreased E2F1 and Myc target gene expression and increased p53 target gene expression. These changes are triggered by the dephosphorylation and stabilization of E2F1 and c-Myc by calcineurin and the activation of *MDM2* transcription by NFAT, which induces p53 degradation.

$Ca^{2+}$ channel inhibitors also induce apoptosis in tumor cells. When hormone-independent and hormone-resistant breast cancer cells are treated with $Ca^{2+}$ channel inhibitors, such as methoxyverapamil and mibefradil, a decrease in intracellular $Ca^{2+}$ concentrations and inhibition of cell proliferation are induced.[89] Furthermore, in breast cancer cells resistant to anti-estrogen therapy used for hormone-dependent breast cancer treatment, a combination of anti-estrogen therapy and $Ca^{2+}$ channel inhibitors suppresses cell proliferation.[90] The inhibition of $Ca^{2+}$/calmodulin signaling, which is activated in gemcitabine-resistant pancreatic cancer, increases the responsiveness to gemcitabine.[91]

Many tumor cells exhibit the aberrant expression of specific $Ca^{2+}$ channels (eg, voltage-dependent $Ca^{2+}$ and TRP channels), which contribute to tumor growth and metastasis. For example, TRPv6 channels are highly expressed in prostate cancer and are considered potential targets for cancer therapy. Because $Ca^{2+}$ channel activation affects cell motility, inhibitors suppress metastasis. In ovarian cancer, the $Ca^{2+}$ channel inhibitor bepridil suppresses cell proliferation, metastasis, and invasion. Transforming growth factor β1-induced epithelial-mesenchymal transition (EMT) is less likely to occur, and EMT markers are reduced. These results have been similarly demonstrated in mouse xenograft experiments.[92]

The relationship between tumor immunity and $Ca^{2+}$ channel inhibitors has also attracted attention in recent years. In colorectal cancer, the $Ca^{2+}$ channel inhibitor nifedipine inhibits the nuclear translocation of NFAT2 and suppresses cancer growth and metastasis in xenograft experiments. Furthermore, suppressing PD-1 and PD-L1 expression also inhibits immune evasion.[93] This finding suggests that $Ca^{2+}$ channel inhibitors may enhance tumor immunity and improve the efficacy of cancer treatment.

Although it is worthwhile to examine the relationship between $Ca^{2+}$ channel blockers and cancer risk, the results have been controversial.[94] Whereas $Ca^{2+}$ channel blockers potentially increase the risk of cancer,[95] others have found no association between $Ca^{2+}$ channel blockers and overall cancer risk.[96,97] Furthermore, in a cohort study on gastric cancer, $Ca^{2+}$ channel inhibitors have been associated with a reduced risk of gastric cancer in patients with hypertension after *Helicobacter pylori* eradication. Longer usage duration and long-acting dihydropyridine are associated with lower risk.[98] These findings depend on complex factors, such as cancer type, duration of drug use, and patient background. The relationship between $Ca^{2+}$ channel blockers and cancer risk remains unclear, and further cohort studies are warranted to clarify these associations.

## CONCLUSION AND PERSPECTIVES

Given the intricate nature of $Ca^{2+}$ signaling, inhibiting certain aspects of it can adversely affect normal cells. It is crucial to identify how localized $Ca^{2+}$ variations initiate specific signals and to design drugs targeting $Ca^{2+}$ signaling pathways that are active only in tumors. Future research should focus on developing precise targeting strategies to enhance therapeutic effectiveness and reduce side effects. Techniques such as $Ca^{2+}$ imaging, signaling analysis, and genetic manipulation should be utilized to thoroughly understand intracellular $Ca^{2+}$ dynamics and their impact on cellular functions.

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## DISCLOSURE STATEMENT

The authors declare no competing interests.

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