Review

Molecular Cancer Research

A Complex Role for Calcium Signaling in Colorectal Cancer Development and Progression

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Abstract

Clinical data suggest that many malignant cancers are associated with hypercalcemia. Hypercalcemia can facilitate the proliferation and metastasis of gastric and colon tumors, and has been considered a hallmark of end-stage disease. However, it has also been reported that dietary calcium or vitamin D supplementation could reduce the risk of many types of cancers. In particular, the intestines can absorb considerable amounts of calcium via Ca^{2+}-permeable ion channels, and hypercalcemia is common in patients with colorectal cancer. Thus, this review considers the role of calcium signaling in the context of colorectal cancer and summarizes the functions of specific regulators of cellular calcium levels in the proliferation, invasion, metastasis, cell death, and drug resistance of colorectal cancer cells. The data reveal that even a slight upregulation of intracellular Ca^{2+} signaling can facilitate the onset and progression of colorectal cancer, while continuous Ca^{2+} influx and Ca^{2+} overload may cause tumor cell death. This dual function of Ca^{2+} signaling adds nuance to the debate over the hallmarks of colorectal cancer, and may even provide new directions and strategies for clinical interventions.

Introduction

Colorectal cancer is usually split into the following types in the clinical diagnosis, as listed in Table 1. To explain the regulation of calcium signals in colorectal cancer progression, this article discusses the association between calcium signals and processes in colon cancer (e.g., the exaggerated cell proliferation, the acquisition of cell migration and invasion capabilities, and the enhanced resistance to cell death and chemotherapy).

Colorectal cancer

Colorectal cancer is a major tumor causing mortality and morbidity in the world, and it is the commonest malignant tumor in Western countries, taking up over 1.2 million of new cases per year (1). In recent studies, a growing number of evidence has shown that the variations in the expression of specific calcium channels or pumps are associated with the occurrence, the growth, as well as the prognosis of colorectal cancer (2–4).

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Calcium signal

Ca^{2+} is a ubiquitous intracellular messenger that controls diverse cellular functions, which can also become toxic and cause cell death. Cell survival is dependent on calcium homeostasis, whereby the intracellular Ca^{2+} is in dynamic equilibrium under the regulation of the plasma membrane, endoplasmic reticulum, and mitochondria. Numerous researches have reviewed and defined the complex elements of calcium signals. The variation of intracellular Ca^{2+} in cells can trigger the expression of proteins or activate pathways and affect tumor cell proliferation, invasion, metastasis, as well as survival (5–8). Most existing studies suggest that an elevated concentration of intracellular Ca^{2+} can promote tumor cell proliferation, and cell death pathways also involve sustained Ca^{2+} increases (Ca^{2+} overload) for excessive calcium entry extracellularly and release from ER store. Accordingly, the function of calcium in the cancer process should be discussed. A large number of ion channels are expressed in the intestinal epithelium for the major function of the colorectum of absorbing water and electrolytes. Numerous studies have reported that the attack of colorectal cancer is often accompanied by abnormal calcium channel protein expression, probably changing the intracelluar calcium concentration and promoting tumor development. Figure 1 provides a common summary of the channels, receptors, calcium store, and proteins that can regulate calcium influx and bind calcium ions (Ca^{2+}), which affect the calcium levels in the cytoplasm of colorectal cancer cells.

Expression of calcium signal in colorectal cancer progress

The dramatic rise in store-operated Ca^{2+} entry (SOCE) and the partial depletion of intracellular Ca^{2+} stores are the most prominent characteristics of Ca^{2+} remodeling in colorectal cancer cells or clinical specimens relative to their normal counterparts (9). SOCE is considered a general Ca^{2+} entry pathway opened by an external stimulus and agonist, including Ca^{2+} release from intracellular stores in the endoplasmic reticulum (ER; refs. 10, 11). It

Table 1. Stage of colorectal cancer in clinical diagnosis

Stage of colorectal cancer	Occurrence tissue	Metastasis
Phase 0	Colonic adenoma or intraductal carcinoma	No metastasis
Phase I	Tumor growing into inner wall of intestine	No metastasis
Phase II	Tumor growing through the intestinal wall to adjacent tissues	Invasion, no lymphatic metastasis
Phase III	Tumor diffusing to lymph nodes	Lymphatic metastasis
Phase IV	Tumor diffusing to liver and lung tissues	Hepatic metastases, pulmonary metastasis
Tumor recurrence	Tumor recurring in colon or other tissues after treatment	Poor prognosis and metastasis

NOTE: According to the invasive and metastasizing area and tissue of cancer cells, the different phases of colorectal cancer are identified by pathological biopsy to definite the appropriate treatments.

has been verified that stromal interaction molecules (STIM) serve as a sensory receptor. The depletion of calcium stores facilitates STIM accumulation at the ER endo-membranes contributing to ER stress and interacts with cation channels in the plasma membrane of the Orai1 and transient receptor potential (TRP) channels. With the activation of ER stress and TRP channels, Ca^{2+} stores are refilled, and the cytoplasmic Ca^{2+} concentration increases, thereby activating downstream effector proteins to regulate colorectal cancer progression (12–14).

Adenosine triphosphate (ATP)ases Ca^{2+} pumps, including sarco/endoplasmic reticulum Ca^{2+}-ATPases (SERCA), plasma membrane Ca^{2+} ATPases (PMCA), and the secretory pathway Ca^{2+} ATPases (SPCA), maintain calcium homeostasis by carrying intracellular Ca^{2+} back to ER or extracellular cells (15–17). In recent years, a growing number of evidence (18) shows that mitochondrial Ca^{2+} uniporter (MCU) and regulatory proteins are vital to bear the Ca^{2+}. Mitochondria also control the SOCE function in colon cancer (19, 20). Excessive ER stress, in collaboration with mitochondria, activates caspase-3 and cell death. Note that MCU, a critical regulator of mitochondrial Ca^{2+} levels, is positively associated with human colon cancer cell death via apoptotic pathways. Mitochondria receive calcium signals from

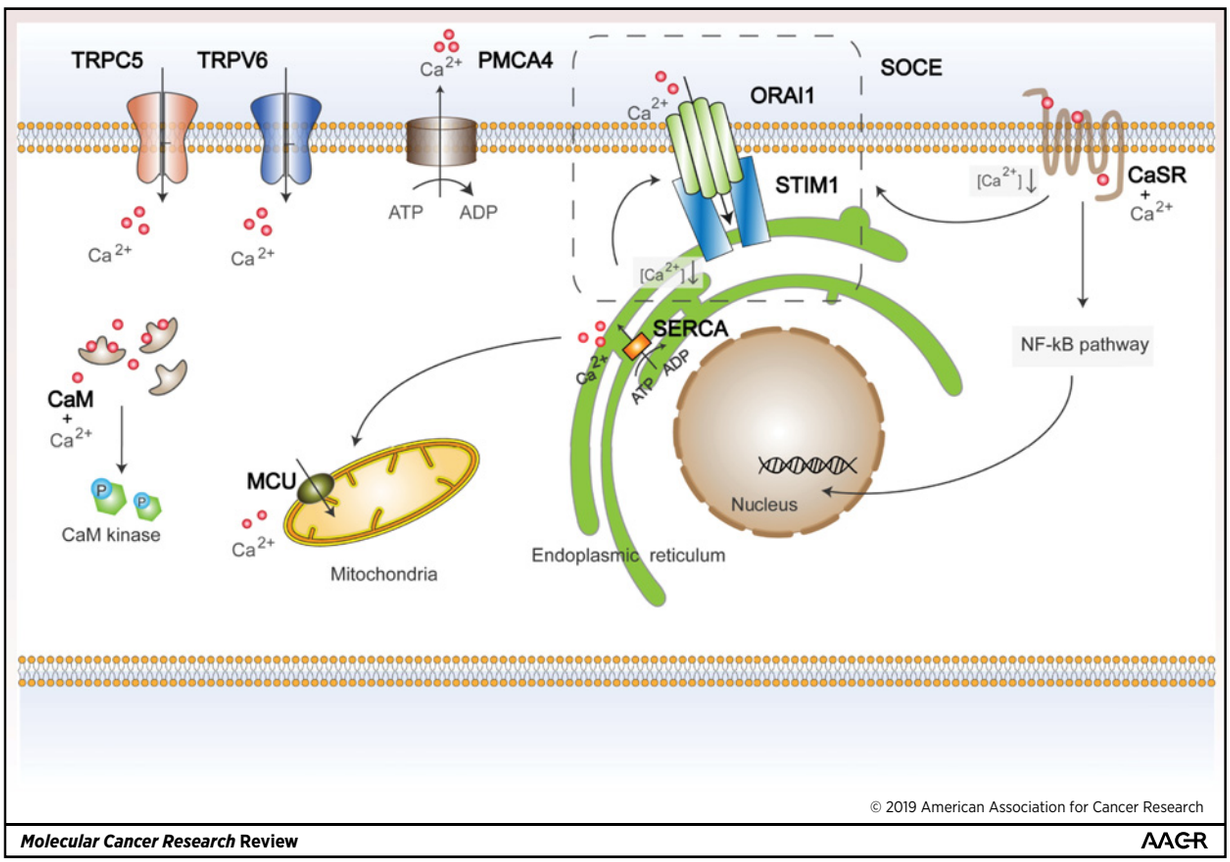

Figure 1.

Specific examples of calcium channels, pumps, and receptors causing calcium changing in colorectal cancer cells. Plasmalemmal Ca^{2+} permeable ion channels are vital for regulating Ca^{2+} influx (e.g., the stimulation of TRPC5 and TRPV6 or the depletion of intracellular Ca^{2+} stores -ORAI1). Ca^{2+} pumps include plasma membrane calcium efflux pumps. For instance, PMCA4 controls the external flow of cells. SERCA refers to the endoplasmic reticulum Ca^{2+} sequestration pumps. ER stress and MCU act as the major repositories of Ca^{2+} in cell. ER is considered the primary regulator of cell death, while the role of mitochondria in apoptosis has been increasingly obvious. CaSR refers to a member of G Protein–coupled receptors.

Table 2. Differential expression of calcium pumps, channels and regulatory in colorectal cancer

Calcium pumps, channels, regulatory	Expression in colorectal cancer tissues/cell lines compared with normal
TRPV6	(24)[a]
TRPC1	(24, 75)[a]
TRPC5	(22, 32, 34, 79)[a]
TRPM2	(76)[b]
TRPM5	(24)[a]
SERCA2	(47)[a]
SERCA3	(47, 60)[a,b]
STIM1	(54, 55)[a]
Orai1	(32)[a]
MCU	(45, 46, 71, 72)[a,b]
CaMKIV	(58)[a]
CaMKII	(78)[a]
CaSR	(42, 51, 87)[a,b]
$CaV_{1.3}$	(59)[a]
PMCA4	(67)[a]

NOTE: Typical expression of calcium signals regulating CRC process in colorectal cancer tissues or cell lines compared with the normal. Note that even a same calcium signal may oppositely express under different pathological conditions to play the corresponding roles.
[a]Increased protein levels in tumor samples.
[b]Decreased protein levels in tumor samples.

the endoplasmic reticulum, and then the MCU intaking Ca^{2+} induces Ca^{2+} overload, thus promoting proapoptosis and cell death in colorectal cancer.

As the primary regulator of Ca^{2+}, TRP channels (21) have been focusing on the feature of colorectal cancer. TRPC5 (22, 23) has been reported to be significantly expressed by TRP channels in colon cancer, which is not only associated with proliferation, invasion, and metastasis, but also affects cells' sensitivity to chemotherapeutics in colorectal cancer. TRPV6 with high Ca^{2+} selectivity and constitutive activity is also overly expressed in human colon cells as compared with normal colonic epithelial cells by transcriptomic analysis of calcium remodeling in colorectal cancer (24).

As mentioned above, the expressions of several calcium pumps and channels are involved in the control of the cancer process and associated disease overall survival and prognosis. Different expressions of Ca^{2+} regulatory channels, pumps, and receptors in colorectal cancer are listed in Table 2, as compared with those of the normal.

Relationship Between Calcium Signal and Colorectal Cancer Process

The physiologic function of the colon is to treat and absorb undigested food, electrolytes, and water. Na^+, K^+, and Ca^{2+} are exchanged by the colon to maintain electrolyte balance (25). Accordingly, ion channels are commonly expressed on the colonic epithelial cell membrane. In the case of colorectal colitis inflammation and electrolyte disturbance, the occurrence of colon cancer may be facilitated. Colonic inflammation is a risk factor for the development of colorectal cancer (26, 27). Chronic intestinal inflammatory diseases (e.g., Crohn disease and ulcerative colitis) often lead to colorectal cancer through a process called colitis-associated carcinogenesis (CAC). As a result, maintaining a balance of electrolytes in the environment of colon epithelial cells is vital to prevent colorectal cancer. Among those electrolytes, Ca^{2+} is the most high-profile ion in colorectal cancer progression, and the effect on cancer growth is controversial.

Complex connection between calcium and colorectal cancer

Lipkin and Newmark (28) first verified that the upregulated concentration of Ca^{2+} in cells regulates colonocyte proliferation by a small trial. However, Garland and colleagues (29) published a contrasting study, showing that high calcium in the diet reduced the risk of colorectal tumors. In recent years, the role of calcium in the tumor cell environment in promoting or inhibiting colon cancer has been controversial. A study reported that calcium intake of more than 1,400 mg/day may reduce the risk of colon cancer, especially in the distal colon (30). A latest report of the World Cancer Research Fund also suggested that high vitamin D intake and calcium supplementation can prevent the development of various tumors. However, in 2018, The Journal of American Medical Association reported that calcium supplements did not significantly enhance survival in patients with cancer, which is the first study to query the chemoprophylaxis of calcium supplements in colorectal cancer (31). Besides, hypercalcemia occurs frequently in the colon microenvironment, thereby facilitating disease progression. As mentioned above, too much or too little Ca^{2+} will not facilitate tumor development.

In some cases, altered Ca^{2+} movement often involves aberrant expressions, cellular localization, and activity of Ca^{2+}-transporting proteins, which contribute to specific tumor characteristics (e.g., unlimited growth, metastasis, and resistance to apoptosis). Stimulation causes Ca^{2+} entry into cells via the TRP channel, contributing to the activation of the HIF-1α/Twist signaling pathway, in subsequent nuclear accumulation of HIF-1α, and ultimately angiogenesis in colon cancer (32). L-type channels mediate Ca^{2+} influx, regulate filopodia stability, and affect cancer cells' motility, facilitating cell invasion and transfer of sensitive downstream integrin signals (33). Besides, Ca^{2+} can upregulate resistance proteins, including ABCB1 (ATP-binding cassette, subfamily B, member 1) and glucose transporter 1 (GLUT1), to escape clinical treatment in colorectal cancer (34).

However, some studies confirm that Ca^{2+} plays an antitumorigenic role in colorectal cancer progression. Ca^{2+} precipitates toxic agents (e.g., secondary bile acids and fatty acids) and then insoluble calcium fatty acids and calcium bile acid are formed and excreted with feces, thereby reducing the risk of colon carcinogenesis (35, 36). Ca^{2+} also regulates the differentiation and apoptosis pathways to accelerate colorectal cancer cell death. The mechanism involves the inhibition of c-Myc, the upregulation of E-cadherin, and the suppression of the canonical Wnt signaling pathway (37, 38). A recent study reported that Ca^{2+} inhibited the expression of replication-licensing factors in a calcium signal control–dependent manner (39). Thus, the association between calcium and the colorectal cancer process is complex, inhibiting or facilitating the development of colorectal cancer via different pathways and calcium signaling.

Calcium signal regulation in colorectal cancer

In recent years, studies on cancer-related molecular mechanisms have gained more insight into the various factors at work in tumor progression. It has been increasingly evidenced that calcium signaling is vital to the progression of colorectal cancer, and it is involved in regulating the characteristics of human colon cancer cells (40). Thus, the calcium signal, rather than calcium

concentration, which regulates the intracellular calcium homeostasis, is considered a crucial factor in colorectal cancer.

However, even the same calcium signaling pathway or protein may have different or opposite regulatory effects in colon cancer development (41, 42). Accordingly, this article discusses some specific examples of colon cancer cells. In the next section, the functions of calcium channels, calcium pumps, and regulatory factors (i.e., proliferation, metastasis, cell death, and drug resistance) in the colorectal cancer process are evaluated and compared with the silencing or inhibition of these calcium signals.

Key Events in Colorectal Cancer Processes Regulated by Calcium Signal

As mentioned above, this article focuses on the critical events in colorectal cancer processes (e.g., proliferation, invasion, metastasis, cell death, and drug resistance) to interpret the different regulations of the calcium signal.

Proliferation

Several TRP channels can affect colorectal cancer cell proliferation. Colon cancer cells with high levels of TRPC5 exhibit fast and strong proliferation, while cells expressing low *TRPC5* gene show downregulated growth *in vitro* and *in vivo* (22). TRPC5 specifically regulates calcium influx. The silence of TRPC5 leads to a reduction in tumor number, size, and proliferation rate in the SW620 colon cancer cell line. The result of the transcriptomic analysis of calcium remodeling in colorectal cancer cell HT-29 and normal colonic epithelial cell NCM-460 shows variations of various TRP channels (e.g., TRPV6, TRPC1, TRPM5, TRPML2, and TRPP2). Among them, TRPV6 is significantly enhanced, controlling and regulating Ca^{2+} influx of cancer cells (24). TRPV6 has been reported to cause a reduction in cancer cell proliferation. SOR-C13, an inhibitor of TRPV6, reduces tumor colony formation *in vitro* (43). TRPM8 is upregulated in HCT-116, while TRPV1 and TRPV2 are downregulated at the mRNA level. Colon carcinogenesis is suppressed by the TRPM8 antagonist in the AOM cancer model and xenograft animal model *in vivo* (44).

The mitochondrial Ca^{2+} uniporter (MCU), an evolutionarily conserved Ca^{2+} channel, exists widely in the inner mitochondrial membrane. The MCU controls intracellular calcium signaling and uptake integrally, being vital to regulate cancer cell growth and aerobic metabolism by acting against the apoptotic pathway under the Warburg effect (45). The receptor-interacting protein kinase 1(RIPK1), a critical signaling molecule in pathways for cell survival, apoptosis, and necroptosis, plays distinct roles in colorectal cancer. RIPK1 can regulate the uptake of mitochondrial Ca^{2+} to promote cell proliferation (46). The MCU significantly upregulates the expression in mitochondria and facilitates tumor cell proliferation via RIPK overexpression in the HT-29 colorectal cancer cell line. The MCU is regarded as a potential and momentous therapeutic target of colorectal cancer.

Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase (SERCA) pumps are encoded by ATP2A, modulating Ca^{2+} transport from the cytoplasm to the endoplasmic reticulum, thus regulating gene expression and cell differentiation. The inhibition of SERCA2 activity induces endoplasmic reticulum stress, leading to G_2–M cycle arrest and growth suppression in SW480 cells *in vitro* and *in vivo* (47). However, some evidence also reveals that SERCA2 and SERCA3 deficiency causing colon cancer occurred with a decrease in cytoplasmic Ca^{2+} concentration (48). Patients with lower SERCA3 expression in colon tumors showed decreased overall survival. Caco-2 with 5-azacytidine treatment led to the upregulation of SERCA3 expression and a decrease in cell viability. The loss of SERCA3 transport ATPase 3 is an early event during the multistep process of colon carcinogenesis. SERCA3 is expressed in normal colon cells, while it is selectively lost in the DLD-1, COLO-205, and Caco-2 colon carcinoma cell lines. The mentioned results suggest that the potential role of SERCA is to maintain the homeostasis of calcium within the cytoplasm and endoplasmic reticulum (47).

The calcium-sensing receptor (CaSR) refers to a member of G Protein–coupled receptors. Because cancer cells lacking the CaSR may not proliferate normally, the chemopreventive effect of dietary calcium pantothenate cannot be exerted on the colonic epithelium to suppress cell proliferation or induce differentiation. Dietary calcium has been demonstrated to inhibit colorectal cancer proliferation in CaSR-positive cancer cells, while it is invalid in CaSR-negative cells, suggesting dietary calcium exerts antitumor properties via CaSR (49). The lack of the CaSR causes a higher accumulation of β-catenin in the nucleus, thereby sustaining activation of the Wnt pathway to promote proliferation in both human colorectal cancer cell lines and CaSR-deficient specimens (50–52). However, some studies verified that the CaSR could activate the noncanonical Wnt pathway, involving the interaction between Wnt5a and its receptor, Ror2, both of which counteract the proliferative signaling of Wnt/β-catenin, recruiting the ubiquitin ligase Siah2 for tumorigenesis (53). Thus, the function of the CaSR in proliferation is bidirectional.

Migration, invasion, and metastasis

The functional units that mediate the SOCE pathway primarily consist of two important molecules, namely the Orai1–calcium ion channels located in the cell membrane and the STIM1-calcium ion receptor located in the endoplasmic reticulum. The feature of STIM1 is to sense the concentration of Ca^{2+}, while Orai1 acts as a channel to perform the SOCE process. In colorectal cancer, Orai1 expression has been considered to respond to hypoxia stimulation, thereby accelerating tumor invasiveness and angiogenesis in HCT-116 and SW480 cells *in vitro*. The SK3 channel, a subset of K^+ and Ca^{2+} ions' channel, hyperpolarized the plasma membrane to increase Ca^{2+} influx via the Orai1 channel–induced SOCE pathway to keep a high Ca^{2+} concentration in the cytoplasm, leading to cancer cell migration and metastasis in HCT-116 *in vitro* (54). Highly metastatic SW620 and LOVO cells have higher STIM1 expression than minimally metastatic SW480 and HT-29 cells. In colorectal cancer patient samples, STIM1 is also overexpressed (74/110). In particular, the samples with positive lymph node metastasis and advanced stages have higher levels of STIM1 expression, suggesting that high STIM1 expression may be positively correlated with colorectal carcinoma metastasis. When STIM1 was overexpressed via transfection with plncx2-STIM1, thapsigargin-induced SOCE increased in DLD-1 cells, as seen through Ca^{2+} imaging. In knockdown STIM1 in DLD-1, SW620, and HCT-116 cells, the effect on EGF-induced migration is suppressed, suggesting that STIM1 is critical for the colon cancer metastasizing process *in vitro* and *in vivo* (55).

Invasion and metastasis have been the major focus of studies that defined the consequences of modifying the calcium signal in

colorectal cancer. The study of TRPC calcium channels in colon cancer cell proliferation is particularly extensive. TRPC5, a receptor-activated nonselective Ca^{2+} channel, is associated with tumor metastasis in patients with colon cancer. Moreover, in colon cancer cell SW620, TrpC5 overexpression causes a robust [Ca^{2+}] rise, downregulates E-cadherin, upregulates mesenchymal biomarker expression, and then facilitates cell migration and invasion, while the colon cancer cell HCT-116, which expresses a lower level of TRPC5, shows a lower ability of invasion and metastasis. However, TRPC1 is highly expressed in HCT-116, which can interact with the Orail 1 channel, thereby promoting Ca^{2+} release and cancer cell migration (32).

Positive or negative adjustment of CaSR expression to adjust the colorectal cancer process in the colon microenvironment remains controversial for the regulation of colorectal cancer processes in the colon microenvironment, but there is growing evidence that the CaSR is vital for colon cancer growth (42). Dietary calcium has been shown to reduce the risk of colon cancer, while exciting the CaSR has been indicated to reduce cancer metastasis. In contrast, hypercalcemia is evident in patients with advanced gastric and colon cancer. The aberrant expression and function of the CaSR make the cells hypersensitive to Ca^{2+}, thereby stimulating the PI3K/AKT pathway to promote migration and invasion (56).

The Ca^{2+}/calmodulin (CaM)-dependent protein kinases (CaMK) are vital for Ca^{2+} regulation after cytoplasmic Ca^{2+} is bound to CaM. Although fewer studies improved the CaMK function on tumor metastasis, CaMKII, one member of the CaMK family, highly appearing in digestive cancers, might affect colorectal cancer progress (57). CaMII is significantly overexpressed in differentiated colon cancer samples compared to paracancerous tissues. CaMKII was suppressed via selective inhibitor KN93, and the capacity of invasion and metastasis of HCT-116 was downregulated. Also, knocking down CaMKII in HT-29 and suppressing CaMKII phosphorylation in SW480 reduces cell invasion (58). CaMKII may be the crucial calcium signaling molecule mediating Ca^{2+} to affect the metastasis of colon cancer.

Besides the above calcium signals, other factors are also considered to be able to affect colon cancer metastasis. Voltage-gated Ca^{2+} channels (CaV) mediate Ca^{2+} influx in excitable cells after plasma membrane depolarization. $CaV_{1.3}$ was found to regulate intracellular calcium concentration and the migration of colon cancer cells via a noncanonical activity. It has been shown via analysis of the Human Protein Atlas that $CaV_{1.3}$, but not $CaV_{1.1}$, $CaV_{1.2}$, $CaV_{1.4}$, is overexpressed in patients with colorectal cancer and α1D expression of $CaV_{1.3}$ promotes migration of HCT116 colon cancer cells *in vitro* (59). Researches showed that SERCA3 expression was negatively associated with lymphatic invasion and the carcinoembryonic antigen (CEA) was higher in patients with colorectal cancer with more positive SERCA3 expression than negative. SERCA3 may act as a prognostic factor for lymphatic metastasis in patients with colon cancer (60).

Key events (e.g., angiogenesis and epithelial–mesenchymal transition) are vital to tumor metastasis progression. Ca^{2+}-independent NOS indicates angiogenesis and colon cancer progression (61). Mechanistic studies revealed that the upregulation of Orai1 by hypoxia potentiates SOCE and then causes activation of the nuclear factor of activated T cells isoform c3 and angiogenesis in colon cancer cells (54). Furthermore, Wnt5a overexpression also induced intracellular calcium and activated noncanonical Wnt/Ca^{2+} signaling in colon cancer to induce epithelial–mesenchymal transition (62). However, the evidence of Ca^{2+}, epithelial–mesenchymal transition and angiogenesis is not exhaustive at present, and more studies are required.

Cell death

As discussed above, calcium signals generally promote the proliferation and metastasis of colorectal cancer. However, disturbing Ca^{2+} influx might cause cell death. Numerous studies have demonstrated that basic and clinical research treatment with chemotherapeutic drugs or new drug ingredients enhances the release of exogenous or endogenous Ca^{2+}, thereby causing cell death in colorectal cancer cells. This is also a major pathway for calcium signaling to play the antitumor role.

The signaling of apoptosis is split into two basic pathways. One is endogenous apoptosis, mediated by death receptors (e.g., TNFα, TRAIL, and FAS-L). The other is exogenous apoptosis caused by enhanced permeability of the mitochondrial outer membrane. Ca^{2+} overload induces apoptosis primarily through endogenous apoptosis. ER is a pluripotent organelle, of which the main functions are protein synthesis and folding. It also serves as a vital location for Ca^{2+} interchange. The mechanism of intracellular Ca^{2+} influx induced by ER stress is complex, involving the common participation of multiple calcium signals. Bortezomib and celecoxib enhance apoptotic cell death by activating the JNK/p38 MAPK pathway and p53 protein to increase ER stress, which increases the concentration of intracellular Ca^{2+}. As a result, the HCT-116 cell undergoes apoptosis (63). Shikonin-induced SNU-407 cell death is mediated by cytoplasmic Ca^{2+} surging by the increased ER stress response. It is generally believed that the process of transporting Ca^{2+} into the ER is maintained by the sarco-endoplasmic reticulum calcium ATPase (SERCA) pump (64). [10-]-gingerol is cytotoxic in several cell types, including human cancer cells (e.g., CRC cell SW480 and HCT15), by a concentration-dependent manner (65). However, the function of [10-]-gingerol has been shown to be abolished by treatment with the SERCA inhibitor thapsigargin and L-type Ca^{2+} channel blockers (66). The specific SERCA inhibitor thapsigargin has also been reported to cause the upregulation of intracellular Ca^{2+} and HCT-116 cell death by depleting endoplasmic reticulum Ca^{2+} stores and ER stress. A specific isoform of the calcium efflux pump-plasma membrane Ca^{2+}-ATPase (PMCA4) interacts with SERCA (67). Some studies suggest that PMCA4 affects the colon cancer cell growth cycle and death. PMCA4 is overly expressed in higher differentiated human colon cancer samples and HT-29, Caco-2 cells. This suggests that PMCA4 affects colorectal cancer cell death (68). PMCA4-knockout cells avoid the decrease in cellular viability and cause a lack of sensitivity to apoptotic stimuli (69).

Because ER and mitochondria are the major repositories of Ca^{2+} in cells, ER is considered the primary regulator of cell death, while the role of mitochondria in apoptosis is increasingly clearer. In recent years, the discovery of the pore-forming subunit of the Mitochondrial Ca^{2+} Uptake Channel (MCU) found a new area for the study of mitochondrial Ca^{2+} regulation and its key role in colon cancer cell death (70). It is generally considered that mitochondria receive calcium signals from ER and decode them into proapoptotic inputs, thereby causing cell death. ER stress–Ca^{2+}-mitochondria signaling induces affluent Ca^{2+} into mitochondria, thereby breaking the calcium homeostasis and the overloading Ca^{2+} and then causing the depolarization of

mitochondria and cell death. Drug stimulation–activated ER and Ca^{2+} into mitochondria contribute to the apoptosis in HT-29 and SW620, which could be reversed by EGTA, a calcium chelator, and reinforced by the ionomycin, an affinity ion carrier of Ca^{2+} (71). Ca^{2+} influx into mitochondria could activate the release of cytochrome *c* and the downstream mediators of apoptosis. The MCU inhibitor CCCP reduces HT-29 cell death, suggesting that Ca^{2+} influx into mitochondria is correlated with cell death (72). The function of MCU-induced apoptosis is suppressed by MCU knockdown by a siRNA in DLD-1 and RKO (70).

Activation of TRP channels through receptor stimulation and enzymes, including the Ca^{2+}-dependent protein phosphatase (e.g., intracellular Ca^{2+} accumulation), is vital to oxidative stress and apoptosis (73, 74). However, the function of promoting cell death of the TRP channel in colorectal cancer has been rarely reported. The expression of TRPC1 is considered necessary for the apoptosis of intestinal epithelial cells. TRPC1 blocker reduces intracellular Ca^{2+} influx and apoptosis in intestinal epithelial cells, whereas the direct effect of TRPC1 on cancer cell apoptosis is not elucidated here (75). TRPM2 has been proven to include apoptosis through cytokine, bacterial peptide activation, and directly oxidative stress. A synergic and comparative effect of 5-fluorouracil (5-FU) and leucovorin (LCV) on Caco-2 has been reported to cause cell death by TRPM2. When using the TRPM2 agonists CMPx, the intracellular Ca^{2+}, ROS, mitochondrial depolarization, caspase 3, and caspase 9 expression become higher in Caco-2 cells under 5-FU and LCV intervention, whereas the effect will be inhibited by the TRPM2 inhibitor ACA (76).

The activity of CaM has been reported to correlate with the phosphorylation of p53 serine residues, thereby ultimately causing cell death. Treatment of HCT-116 stimulates the entering of extracellular Ca^{2+} via long-lasting–type plasma membrane channels, thereby causing the Fas-associated protein to exhibit death domain (FADD) expression in plasma membranes, as well as exogenous apoptosis (77). Some drugs promote colon cancer cell apoptotis via AMPK activation, and AMPK activation has been shown to be mediated by CaMKIV phosphorylation. Using STO-609 to suppress phosphorylation of CaMKIV in HT-29 contributes to the phosphorylation of AMPK and abolishing of cell death (78).

Drug resistance

As mentioned above, 5-FU is commonly used in chemotherapy of colorectal cancer, which makes cells sensitive to calcium signals, thus hindering proliferation and reducing cell mortality. However, drug resistance and poor prognosis often occur during 5-FU treatment. The only calcium-permeable ion channel that has been studied in the context of 5-FU therapeutic resistance in colorectal cancer is the TRPC5 channel. The results of *in vitro* experiments suggested that the mRNA and protein expression levels of TRPC5 in HCT-8 and LoVo cells treated with 5-FU were higher than those of untreated cells. In the samples of 5-FU chemotherapy in 72 patients with colorectal cancer, 44 patients became nonresponsive following two cycles of drug treatment. In particular, 21 of the 44 nonresponders showed TRPC5 overexpression. TRPC5 might be regarded as a symbol of 5-FU drug resistance in colorectal cancer, which regulates the expression of downstream drug resistance proteins, for example, the ATP-binding cassette subfamily B1 (ABCB1) and glucose transporter 1 (GLUT1; refs. 34, 79). TRPC5 silencing in 5-FU–resistant HCT-8 cells can suppress ABCB1 and GLUT1 expression and increase the sensitivity to drugs. Some studies have proved that cancer cells are nonsensitive to drugs via activation of the Ca^{2+}-dependent transcription factor NFAT with elevated TRPC5, thereby enhancing multidrug resistance (MDR)-ATPase 1 transcription and forming a positive feedback loop to enhance drug resistance. La3+-elicited Ca^{2+} rising in HCT-8/5-Fu and LoVo/5-Fu cells can be suppressed by T5E3, a specific blocking antibody of TRPC5. Calcium influx mediated by TRPC5 is considered a key factor in the occurrence of drug resistance.

As mentioned above, since the endoplasmic reticulum maintains the calcium homeostasis in the cytoplasm, ER stress induces cell death via increased intracellular Ca^{2+}. Sorcin, a mitochondrial isoform, has been reported to participate in antiapoptosis and MDR in multiple tumor types by binding to Ca^{2+} (80–82). It has been observed that Sorcin is directly correlated with MDR1 expression and is vital to inducing the MDR phenotype in leukemia and gastric cancer (83). Several studies have indicated that both 18- and 22-kDa isoforms of Sorcin participate in the regulation of drug resistance by preventing ER stress in colon cancer cells (84). The 22-kDa isoform of Sorcin is upregulated at a high Ca^{2+} concentration in ER to develop resistance to 5-FU, oxaliplatin, and irinotecan (85). In such a way, loperamide overcomes the resistance of bortezomib by enhancing the ER stress and ER dilation, which leads to the accumulation of misfolded proteins and disturbs the calcium homeostasis, which is conducive to subsequent paraptosis-like cell death in human colon cancer cells (86).

Another system in balancing calcium homeostasis reported to affect MDR in colorectal cancer is CaSR. The activation of CaSR by agonists or extracellular stimulation enhances the sensitivity of colon cancer cells to mitomycin C and fluorouracil (37, 87). According to the early discussion of the CaSR function in colorectal cancer proliferation, metastasis, and cell death, CaSR expression affects colon cancer cell differentiation. The degree of differentiation in colon carcinomas can affect survival and modulate cellular sensitivity to chemotherapy (88, 89). The CaSR has been reported to be able to upregulate the expression of the mitomycin C–activating enzyme NAD(P)H: quinone oxidoreductase1(NQO-1) and to downregulate the expression of 5-FU drug targets, thymidylate synthase (TS) and the antiapoptotic protein surviving in SW480 cells (90).

Conclusion

The unique environment of colorectal cancer with hyperelectrolyte and hypercalcemia makes various calcium signals express and plays a regulatory role. In this study, as listed in Table 3, we find that calcium signals may promote or inhibit the colorectal cancer process. The adjustment of the same calcium regulation varies with the phase of colorectal cancer progression. As a result, the function of the calcium signal is found to be able to maintain the Ca^{2+} balance of cancer cells, and calcium homeostasis is conducive to colorectal cancer prevention. However, under pathologic conditions, especially during cell invasion and metastasis, calcium channels and pumps are activated, and Ca^{2+} influx in abundance continues to deteriorate in subsequent cancer. In brief, the progression of Ca^{2+} to the colorectum is bidirectional. It is summarized as follows: elevation of intracellular Ca^{2+} can improve the proliferation, migration, invasion, metastasis, and drug resistance of colorectal cancer cells, which are often

Table 3. Results of silencing/knockdown of calcium signaling regulators in colon cancer

	Calcium signaling regulator	Outcome of silencing/knockdown			
		Proliferation	Metastasis	Cell death	Resistance
TRP	TRPC5	(22)[a]	(32)[a]		(34, 79)[a]
	TRPC1	(24)[a]	↓	(75)[a]	
	TRPV6	(24, 43)[a]			
	TRPM2			(76)[a]	
	TRPM8	(44)[a]			
	TRPV1	(44)[b]			
	TRPV2	(44)[b]			
Mitochondrial Ca^{2+} regulators	MCU	(45, 46)[a]		↓(70–72)	
SERCA	SERCA2	(47, 48)[a,b]			
	SERCA3	(47)[a]	(60)[b]		
Calcium-sensing Receptor	CaSR	(49–53)[a,b]	(42, 56)[a,b]		(37, 87–90)[a,b]
SOCE	STIM1		(54, 55)[a]		
	Orai1		(32)[a]		
CaM-dependent protein kinases	CaMKII		(58)[a]		
	CaMKIV			(78)[a]	
Voltage-gated Ca^{2+} channels	$CaV_{1.3}$		(59)[a]		

NOTE: Calcium signals include cell membrane and intracellular regulators. TRP channels, the major substance on the cell membrane that regulates calcium influx, are vital to develop tumor cells. However, some calcium signals regulate tumors in both directions (e.g., SERCA and CaSR), which significantly maintains the balance of calcium ions.
[a]Decreased.
[b]Increased.

associated with the high expression and the abnormal activation of calcium channel proteins in cells. However, when intracellular Ca^{2+} rises to a certain level (Ca^{2+} overload), it will cause depolarization, apoptosis, or some other biological processes of cells, ultimately leading to cancer cell death. As mentioned above, intracellular Ca^{2+} overload can cause tumor cell death; however, the extent of Ca^{2+} that can cause cell death is difficult to scope, and excessive Ca^{2+} may cause the death of normal cells as well. On these bases, pharmacologic inhibitors or siRNA-mediated effects on calcium channels may inhibit the occurrence and the deterioration of colorectal cancer. However, not many of the pharmacologic agents acting on calcium channels can be taken through the complete drug development process. Thus, developing antitumor drugs targeting calcium signaling remains rather challenging. Furthermore, the intracellular Ca^{2+} of other stromal cells may also vary when the cancer cells are being targeted, so the overall biological effects should be assessed before possible clinical uses.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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