Chapter 19
Remodeling of Calcium Entry Pathways in Cancer

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Abstract Ca^{2+} entry pathways play important roles in control of many cellular functions, including long-term proliferation, migration and cell death. In recent years, it is becoming increasingly clear that, in some types of tumors, remodeling of Ca^{2+} entry pathways could contribute to cancer hallmarks such as excessive proliferation, cell migration and invasion as well as resistance to cell death or survival. In this chapter we briefly review findings related to remodeling of Ca^{2+} entry pathways in cancer with emphasis on the mechanisms that contribute to increased store-operated Ca^{2+} entry (SOCE) and store-operated currents (SOCs) in colorectal cancer cells. Finally, since SOCE appears critically involved in colon tumorogenesis, the inhibition of SOCE by aspirin and other NSAIDs and its possible contribution to colon cancer chemoprevention is reviewed.

Keywords Store-operated Ca^{2+} entry • Cancer • Colorectal cancer • Aspirin • NSAID

19.1 Intracellular Ca^{2+} Homeostasis and Ca^{2+} Entry Pathways

Intracellular Ca^{2+} is a very versatile second messenger involved in the control of many different physiological and cellular processes in the short and the long-term. Unlike other messengers, Ca^{2+} is not created or destroyed by cells, but transported down electrochemical gradients through specific channels, or transported back against gradients at the expense of the energy stored as ATP (Ca^{2+} pumps) or coupled with transport of other ions (Ca^{2+} exchangers). Ca^{2+} channels, pumps and exchangers are located in the plasma membrane and endomembranes of the endoplasmic reticulum (ER), mitochondria and other cell organelles. Unlike other ions,

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the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) is so little (100 nM) that even minimal changes in channel activity may induce large increases in $[Ca^{2+}]_{cyt}$. Thus, this cation is unique in the sense that has been selected by nature to carry signals inside cells and organelles. Changes in $[Ca^{2+}]_{cyt}$ may be restricted in time and space resulting in elementary events or Ca^{2+} microdomains that can regulate specifically located cellular functions such as exocytosis in plasma membrane, cell respiration and ATP synthesis in mitochondria or gene transcription in the nucleus. Alternatively, elementary events may give rise to regenerative waves leading to sustained, global changes associated to long-term events like cell growth, differentiation or death. Thus, the study of the intracellular Ca^{2+} homeostasis is a matter of ion transport across boundaries that requires the use of sophisticated methodologies for recording Ca^{2+}-driven currents (patch-clamp) or measuring the tiny concentrations of Ca^{2+} in the cytosol or the very variable Ca^{2+} concentrations in organelles and/or subcellular environments using live cell imaging and targeted calcium probes with different affinities for Ca^{2+}.

Ca^{2+} pumps and transporters contribute significantly to the maintenance of resting $[Ca^{2+}]_{cyt}$ and to the recovery of basal $[Ca^{2+}]_{cyt}$ after stimulation. However, most increases in $[Ca^{2+}]_{cyt}$, are rather due to activation of Ca^{2+} entry pathways at the plasma membrane and Ca^{2+} release channels at the ER. In the RE, IP_3 receptors and ryanodine receptors are ligand-gated Ca^{2+} channels that mediate Ca^{2+} release from stores. Ca^{2+} release channels induce transient increases in $[Ca^{2+}]_{cyt}$ but their activity may secondarily activate Ca^{2+} channels in plasma membrane that are gated by the filling state of Ca^{2+} stores. In mitochondria, the main Ca^{2+} channel is the so-called mitochondrial Ca^{2+} uniporter (MCU), a Ca^{2+}-activated Ca^{2+} channel recently characterized at the molecular level [1, 2]. Activation of this channel in physiological conditions leads to mitochondrial Ca^{2+} uptake and removal of Ca^{2+} from cytosol. This is due to the fact that mitochondria inner membrane shows a strong mitochondrial potential, negative inside the mitochondrial matrix, thus favoring Ca^{2+} influx into mitochondria provided that cytosolic Ca^{2+} is large enough to activate the MCU [3, 4]. At the plasma membrane there are many different types of Ca^{2+} channels, including receptor-operated and voltage-operated Ca^{2+} channels that are widely expressed in excitable cells (ROCCs and VOCCs) together with voltage-independent channels that are particularly relevant in non-excitable cells. The most important Ca^{2+} entry pathway in non-excitable cells is the store-operated Ca^{2+} entry (SOCE), a Ca^{2+} entry pathway ubiquitous and responsible for the entry of Ca^{2+} after agonist-induced activation of phospholipase C and emptying of intracellular Ca^{2+} stores.

SOCE is activated physiologically after the emptying of the intracellular Ca^{2+} stores induced by physiological agonists producing IP_3 or, pharmacologically, after inhibition of the sarcoplasmic and endoplasmic reticulum Ca^{2+} ATPase (SERCA) pump with thapsigargin or similar SERCA antagonists. SOCE usually remains active until Ca^{2+} stores become filled again. Interestingly, SOCE is also regulated by mitochondria. Mitochondrial control of SOCE is due to the ability of these organelles to take up Ca^{2+}, thus preventing refilling of Ca^{2+} stores and preventing also the Ca^{2+}-dependent inactivation of IP_3 receptors and the own Ca^{2+} release activated channels responsible for SOCE. This mechanism ensures the efficient emptying of

Ca^{2+} stores and SOCE activation and is believed to be critical to maintain Ca^{2+} entry in those signaling pathways in which a sustained activation is required. This is the case, for instance, of the activation of the nuclear factor of activated T (NFAT) cells during the immunological synapsis. In this case, a sustained entry of Ca^{2+} through SOCE is needed to promote IL2 gene expression and the clonal expansion of the activated T cell [5].

For years, the molecular basis of SOCE had remained elusive. However, the molecular players involved in SOCE began to be crack after the discovery of the TRP superfamily of ion channels. Some TRP channels were held during quite some time responsible for SOCE. However, more recently, the protein families STIM and ORAI were described to be the cornerstone of SOCE becoming now fully accepted as responsible for Icrac and SOCE in multiple cell types. At the molecular level, SOCE begins with the emptying of intracellular Ca^{2+} stores. Emptying, in this case, means that Ca^{2+} concentration inside the ER decreases from around 700 μM before stimulation to about 200 μM after agonist-induced Ca^{2+} release, as revealed by ER targeted probes with very low affinity for Ca^{2+} [6]. This "emptying" is detected by a sensor named Stromal Interaction Molecule 1 (STIM1) which, upon dissociation of Ca^{2+} ions from Ca^{2+} binding sites, undergoes oligomerization and its interaction with ORAI1, a Ca^{2+} channel located in specific places of the plasma membrane. This interaction opens Ca^{2+} specific CRAC channels, thus enabling the entry of Ca^{2+} into the cytosol. The whole mechanism is reversed when Ca^{2+} stores become filled again [7–9]. There is another Ca^{2+} sensor at the ER called STIM2, which has a lower affinity for Ca^{2+} and is believed to be activated only after moderate depletion of Ca^{2+} stores [10]. However, its role in SOCE remains controversial. In addition, two other ORAI family members (ORAI2 and ORAI3) may also be involved in SOCE but their role is also poorly known [11]. Finally, other types of channels, including some of the TRP superfamily, particularly TRPC channels may contribute to SOCE as well by forming channel complexes with STIM1 and ORAI1 or simply forming alternative store-operated channels less selective for Ca^{2+} [12, 13].

Other Ca^{2+} entry pathways also widely expressed are now collectively termed as store-independent Ca^{2+} entry (SICE) pathways. They are less known and most of them are characterized only at the functional level but not at the molecular level. They include channels gated by araquidonic acid, diacylglycerol (DAG) and stretch activated channels. Most of these channels may well be mediated by TRP channels and/or channel complexes made thereof. Other chapters in this issue describe in more detail characteristics and molecular basis related to store-independent Ca^{2+} entry pathways.

19.2 Ca^{2+} Entry Remodeling in Cancer

A series of recent reports suggest that changes in intracellular Ca^{2+} homeostasis (remodeling) may be critically involved in various forms of cancer. This is not surprising if we consider that the so many cellular processes exacerbated in the

Table 19.1 Changes in expression levels of voltage-operated Ca^{2+} channels [VOCCs] in tumor cells relative to normal cells or tissue samples

Channel isoform	Type of tumor	Research model	Change	Reference
$Ca_v1.1$	Colorectal	Cell lines and tissue samples from patients	↑ mRNA	[20]
$Ca_v1.2$	Colon	Cell lines and tissue samples from patients	↑ mRNA	[21]
$Ca_v3.1$	Colorectal	Cell lines and tissue samples from patients	↓ mRNA	[22]
	Gastric	Cell lines and tissue samples from patients	↓ mRNA	[22]
$Ca_v3.2$	Prostate	Tissue samples from patients	↑ Protein	[23]

transformed cell such as excessive cell proliferation, migration and invasion capabilities as well as apoptosis resistance and cell survival are regulated by intracellular Ca^{2+} [14–16]. A Ca^{2+} entry pathway that has been implicated in cancer is SOCE [17, 18] which could offer new therapeutic possibilities against cancer as suggested earlier [19]. However, some other Ca^{2+} entry pathways and channels have been also related to tumorogenesis. For example, it has been reported that voltage-gated Ca^{2+} channels (VOCCs) are overexpressed in various types of tumors (Table 19.1).

Thus, epithelial tumors express L-type [21] and T type VOCCs and their inhibition may prevent cell proliferation [24]. It is not clear the role of VOCCs in epithelial cells or even if they may work as channels. For example, plasma depolarization with medium containing a high concentration of K^+, a typical experimental maneuver intended to activate voltage-gated Ca^{2+} entry in Ca^{2+} imaging experiments in excitable cells [25], has no effect in epithelial cells, either normal or tumoral. Accordingly, increased expression of VOCCs in cancer cells may not be related to intracellular Ca^{2+} homeostasis. Expression of other Ca^{2+} channels of the TRP superfamily, particularly canonical TRP channels not so selective for Ca^{2+}, has been reported as well to be altered in different tumors (Table 19.2). Other channels of the same superfamily have been also reported to be either overexpressed or downregulated in different tumors, particularly TRPV4, TRPV6 and TRPM8 in carcinomas and other tumors [34, 37, 38]. The role of these channels in cancer remains unclear but recent evidence suggests a role for some of these channels in cancer hallmarks. For instance, it has been shown that the calcium selective channel TRPV6 is able to translocate to the plasma membrane via Orai1-mediated mechanism controlling cancer cell survival [39]. This expanding issue is being covered by excellent recent reviews [40]. See Table 19.3 for further details on changes in the expression of these channels in different tumors.

More recently, it has proposed an essential role for SOCE and/or its molecular players in tumorigenesis. For example, STIM1 and Orai1 may be critical for migra-

Table 19.2 Changes in expression levels of TRPC channels in tumor cells relative to normal cells or tissue samples

Channel isoform	Type of tumor	Research model	Change	Reference
TRPC1	Breast	Tissue samples from patients	↑ mRNA and protein	[26]
TRPC3	Ovary	Tissue samples from patients	↑ Proteína	[27]
	Breast	Tissue samples from patients	↑ mRNA	[28]
TRPC4	Kidney	Cell lines	↓ mRNA	[29]
TRPC6	Esophagus	Tissue samples from patients	↑ mRNA and protein	[30, 31]
	Gastric	Tissue samples from patients	↑ mRNA and protein	[32]
	Glioma	Tissue samples from patients	↑ mRNA and protein	[33]
	Liver	Tissue samples from patients	↑ mRNA and protein	[34]
	Breast	Tissue samples from patients	↑ mRNA and protein	[26, 28]
		Cell lines and Tissue samples from patients	↑ mRNA and protein	[35]
	Prostate	Cell lines	↑ mRNA	[36]

tion of breast cancer cells and metastasis [27]. STIM1 plays an important role in cell growth and migration in cervical cancer [54]. Indeed STIM1 is overexpressed in 70 % of cervical cancers, which has been associated with an increased risk of metastasis. As a matter of fact, the suppression of STIM1 inhibits human glioblastoma cell proliferation and induces G0/G1 phase arrest [55]. Moreoever, STIM1 and Orai1 mediate CRAC channel activity and are essential for human glioblastoma invasion [56]. Orai1 could be also overexpressed in breast cancer [57]. The entry of Ca^{2+} mediated by Orai1 regulates also proliferation and survival in glioblastoma cells and hepatoma [34].

The role of other molecular players involved in SOCE in tumorogenesis is unknown except for the case of Orai3 that has been proposed to be an estrogen receptor α-regulated Ca^{2+} channel that promotes tumorigenesis [58]. Likewise, Orai3 has been also reported to constitute a native SOCE regulating non-small cell lung adenocarcinoma cell proliferation [59]. Therefore, multiple recent evidence suggests an unexpected role for intracellular Ca^{2+} remodeling, particularly SOCE in cancer [15, 16]. See Table 19.4 for further details on changes in expression of molecular players involved in SOCE in different forms of cancer.

Table 19.3 Changes in expression of TRPV4, TRPV6 and TRPM8 in tumor cells relative to normal cells or tissue samples

Channel isoform	Type of tumor	Research model	Change	Reference
TRPV4	Skin	Tissue samples from patients	↓ mRNA and protein	[41]
	Bladder	Tissue simple and mouse cell line	↓ mRNA and protein	[42]
TRPV6	Breast	Tissue samples from patients	↑ mRNA and protein	[26, 37, 43]
	Prostate	Tissue samples from patients	↑ mRNA	[44, 45]
		Tissue samples from patients	↑ Proteína	[37]
	Thyroid	Muestras de tejido de pacientes	↑ Proteína	[37]
	Colon	Tissue samples from patients	↑ Proteína	[37]
	Ovary	Tissue samples from patients	↑ Proteína	[37]
	Lung	Tissue samples from patients	↓ mRNA and protein	[46]
TRPM8	Pancreas	Cell lines and tissue samples from patients	↑ mRNA and protein	[47]
	Prostate	Tissue samples from patients	↑ mRNA and protein	[48–51]
	Androgen-independent prostate tumor	Tissue samples from patients	↓ mRNA	[51]
	Breast	Tissue samples from patients	↑ mRNA and protein	[26, 48]
		Cell lines and tissue samples from patients	↑ mRNA and protein	[52]
		Tissue samples from patients	↑ mRNA and protein	[48]
		Tissue samples from patients	↑ mRNA	[48]
		Cell lines and tissue samples from patients	↑ Protein	[53]
	Colorectal	Tissue samples from patients	↑ mRNA	[48]
	Lung	Tissue samples from patients	↑ mRNA	[48]

Table 19.4 Changes in expression of ORAI and STIM family members in tumor cells relative to normal cells or tissues

Channel isoform	Type of tumor	Research model	Change	Reference
ORAI1	Breast	Cell lines	↑ mRNA	[57]
	Glioblastoma	Cell lines and tissue samples from patients	↑ mRNA	[56]
	Glioma	Tissue samples from patients	↑ Protein	[60]
	Melanoma	Cell lines	↑ Protein	[61]
	Kidney	Tissue samples from patients	↑ Protein	[62]
ORAI3	Breast	Cell lines and tissue samples from patients	↑ mRNA	[63]
	Prostate	Tissue samples from patients	↓ mRNA	[64]
		Tissue samples from patients	↑ mRNA	[65]
STIM1	Glioblastoma	Tissue samples from patients	↑ mRNA	[66]
	Cervix	Tissue samples from patients	↑ protein	[54]
	Breast	Cell lines	↑ mRNA	[57]
	Lung	Tissue samples from patients	↑ protein	[55]
	Liver	Cell lines and Tissue samples from patients	↑ mRNA	[27]
	Melanoma	Cell lines	↑ protein	[61]
	Colon	Tissue samples from patients	↑ mRNA and protein	[21]
STIM2	Glioblastoma	Tissue samples from patients	↑ mRNA	[67]
	Colon	Tissue samples from patients	↑ mRNA	[68]

19.3 Ca^{2+} Entry Pathways in Normal and Colon Cancer Cells

We have recently reported a deep remodeling of SOCE in colorectal cancer [69]. For investigating Ca^{2+} remodeling in colon cancer we have compared Ca^{2+} entry pathways in a series of normal human colonic mucosa cell lines and human colon adenocarcinoma cells. We found that SOCE is significantly larger in colorectal cancer cells than in normal cells [69]. Both normal and tumor cells differed also in their rate of cell proliferation with tumor cells showing always larger rates of cell proliferation. Interestingly, there was a clear correlation between SOCE and the rate of cell proliferation suggesting that the larger rate of cell proliferation of tumor cells follows changes in SOCE [69]. Consistently, SOCE inhibition with antagonists prevents not only tumor cell proliferation but also cell invasion as tested by Matrigel invasion assays. Therefore, enhancement of SOCE in colon cancer cells may contribute not only to increased cell proliferation characteristic of tumor cells but also to cell invasion, both being critical hallmarks of cancer [69].

Differences between normal and tumor cells regarding Ca^{2+} entry and Ca^{2+} release induced by physiological agonists were also studied in detail. ATP and

Carbachol, two physiological agonists that activate G protein-coupled receptors and phospholipase C, increase $[Ca^{2+}]_{cyt}$ in both normal and tumor cells. However, the rises in $[Ca^{2+}]_{cyt}$ are much larger in tumor cells compared with normal colonic epithelium cells. Analysis in Ca^{2+} free medium revealed that the two agonists released more Ca^{2+} in tumor cells than in normal cells. Results on agonist-induced Ca^{2+} entry were surprising: In fact, both agonists induced Ca^{2+} entry only in tumor cells but not in normal cells, despite that both released Ca^{2+} from intracellular stores. To avoid contribution of possible differences in expression of GPCRs in normal and tumor cells, experiments were carried out using caged IP_3. Again, the IP_3-induced increase of $[Ca^{2+}]_{cyt}$ was larger in tumor cells than normal cells [69]. However, these results could be due to either differences in the level of expression and/or activity of IP_3 receptors and/or to differences in the extent of Ca^{2+} store content. Experiments using ionomycin or cyclopiazonic acid in Ca^{2+} free medium revealed that Ca^{2+} stores were paradoxically larger in normal cells than in tumor cells. In other words, Ca^{2+} stores in tumor cells are partially empty. These data together with the data derived form Ca^{2+} release experiments, always much larger in tumor cells, suggest that in tumor cells Ca^{2+} stores are nearly depleted and any minimal stimulation is sufficient to reach the threshold for SOCE activation. However, in normal cells, where Ca^{2+} stores are full and physiological stimulation releases only a limited amount of Ca^{2+}, SOCE threshold is beyond reach leading to no Ca^{2+} entry. These data may explain why agonists do activate Ca^{2+} entry only in tumor cells but not in normal mucosa cells [69]. Of course, we have to take into account that these data derive from the analysis of a few, non isogenic cell lines serving as models of normal and tumor colon cells. Results must be confirmed in additional normal and tumor samples derived from the same specimen.

19.4 Store-Operated Currents in Normal and Colon Cancer Cells

To identify functional and pharmacologically SOCs in normal and tumor cells we have used planar patch-clamp in the voltage clamp configuration [69]. The depletion of Ca^{2+} stores with thapsigargin in normal colonocytes induces a small, voltage-independent, inward rectifying current that is highly selective for Ca^{2+} and sensitive to La^{3+} and to low concentrations of 2-APB [69]. Therefore, this current is very similar, if not identical, to the Icrac current originally described as responsible for SOCE in mast and T cells. In tumor cells, however, SOCs are very different. Emptying of Ca^{2+} stores induces two types of currents: First, a current similar to Icrac recorded in normal cells with the only exception that current density is larger in tumor cells. Second, we observe a different Isoc, absent in normal cells, with a large outward component, not selective for Ca^{2+} and sensitive to high concentrations of 2-APB [69]. The data suggest that SOCE in normal cells is mediated by Orai1 and STIM1, the molecular players previously reported to be involved in Icrac in

mast and T cells. However, tumor cells would have two streams, one should be Icrac but with larger current density probably mediated by changes in expression of Orai1 and Stim1. The other current could be mediated by a TRP channel. Accordingly, data suggest that store-operated currents may be mediated by different channels in normal and tumor cells.

19.5 Molecular Basis of Remodeling of Ca^{2+} Entry Pathways in Colorectal Cancer

Molecular candidates involved in SOCE and SOCs in normal and colon cancer cells have been investigated using conventional and quantitative RT-PCR. All members of the STIM and Orai families (Orai1, 2, 3 and STIM1 and 2) are expressed in normal and tumor cells. Other TRPC channels including TRPC1 and TRPC4 channels are expressed as well in both normal and tumor cells. However, other genes that have been related to SOCE are expressed only in normal cells but not in tumor cells including TRPV6 and TRPM8 suggesting a loss of function during tumorogenesis. Finally, some other related channels are missing in both cell types. Quantitative RT-PCR and Western blotting show significant increases in the expression of many of the above genes. Interestingly, the expression pattern of the different molecular players involved in SOCE and SOCs is roughly similar in normal and tumor cells except that most genes are rather increased in tumor cells relative to normal cells. At the protein level, Orai1, Orai2, and Orai3 proteins are increased significantly in tumor cells. Similar results are obtained with TRPC1 and STIM1. Surprisingly, although STIM2 gene expression is increased in tumor cells, STIM2 protein is nearly lost in tumor cells [69].

Using siRNA against each of these specific molecular players, we have established that SOCE and SOCs in normal cells are mediated by the interactions between ORAI1, STIM1 and STIM2. However, in tumor cells, SOCs and SOCE are more complex and include not only STIM1 and Orai1 but also TRPC1. The increased expression of both may contribute to explain the increase in SOCE and agonist-induced Ca^{2+} observed in tumor cells relative to normal cells (Fig. 19.1).

Therefore, SOCs in colon carcinoma cells are made of different molecular players than in colon normal mucosa cells. Differences involve likely changes in channel complexes including the appearance of TRPC1 and the disappearance of STIM2 from those complexes. This view is supported by silencing experiments. TRPC1 silencing has no influence on Icrac in normal cells but it decreases not only the outward component associated to the non-selective current present only in tumor cells but also the inward component. Moreover, ORAI1 silencing decreases Icrac in normal cells and both components inward and outward in tumor cells. Thus, these data suggest strongly that ORAI1 and TRPC1 form a channel complex in tumor cells but not in normal cells. The data invite speculation as to what is the role played by TRPC1 in SOCE. Silencing experiments reveal that TRPC1 knockdown does not

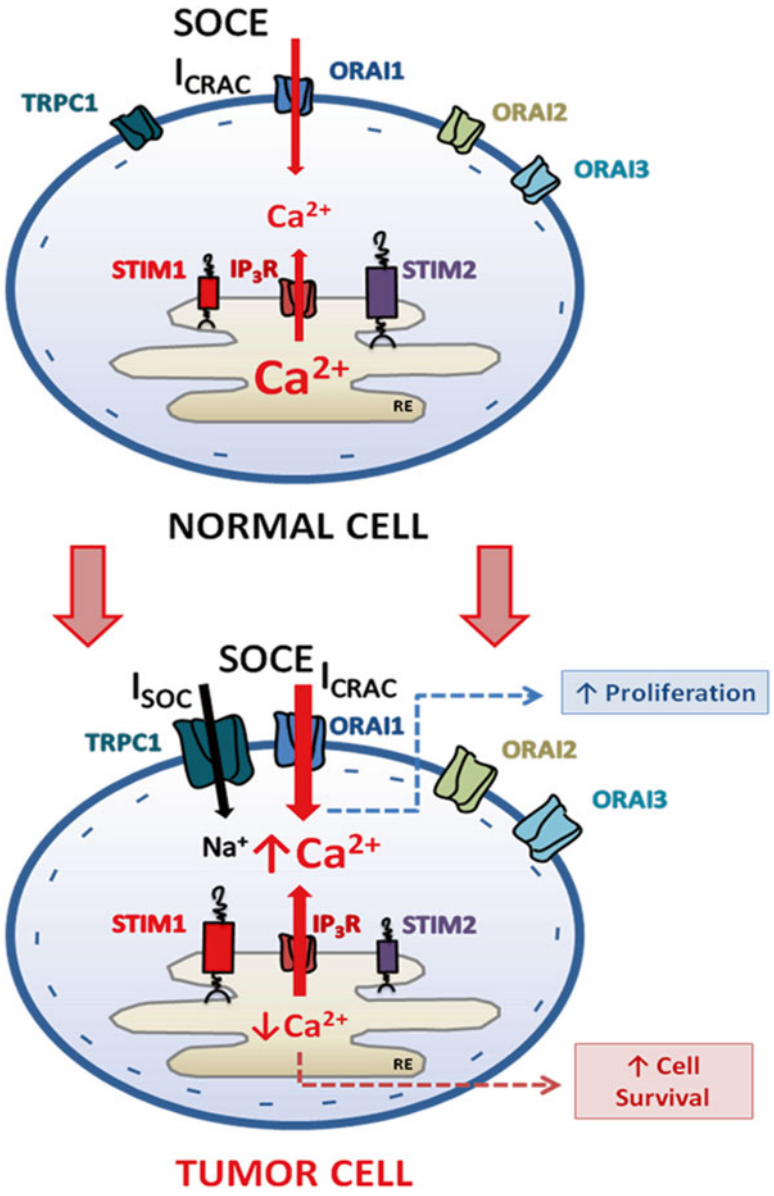

Fig. 19.1 Ca^{2+} entry remodeling in colorectal cancer. Normal human colon cells (*above*) show a small SOCE mediated Orai1 and STIM1 and a large Ca^{2+} store content associated with the large expression of STIM2. The cells also express TRPC1, TRPC4, TRPC7, TRPV6 and TRPM8. Cells of human colon adenocarcinoma (*below*) present large and modified SOCE mediated by increases in expression of Orai1, TRPC1 and STIM1, and partial depletion tank contents mediated decreased expression of STIM2. These cells lack TRPC7, TRPV6 and TRPM8. Remodeling may contribute to increased proliferative capacity, invasion and survival of tumor cells [69]

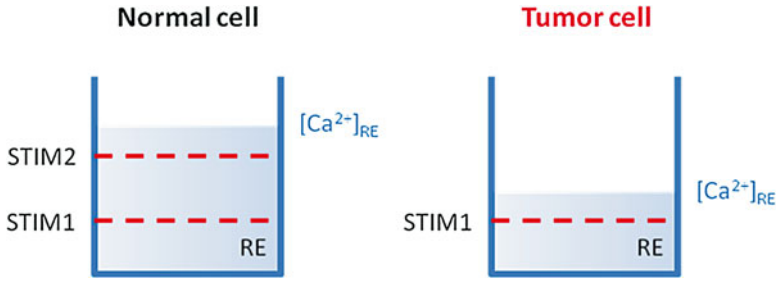

Fig. 19.2 Loss of Stim2 in tumor cells decreases Ca^{2+} store content in tumor cells. Normal cells express both Stim1 and Stim2, ER Ca^{2+} sensors with different affinities for Ca^{2+}. Loss of Stim2 in colon cancer cells leaves Stim1 as the only sensor available that refills Ca^{2+} stores poorly leading to partially depleted stores in tumor cells

decrease SOCE induced by thapsigargin in tumor cells. Therefore, TRPC1 might play roles different from supporting Ca^{2+} entry in tumor cells. Since this channel permeates mostly Na^{+}, a possible role could be to depolarize plasma membrane and limit Ca^{2+} entry. Alternatively, TRPC1 may be involved in changes in cell volume related to cell cycle.

What is the biological significance of STIM2 loss in tumor cells? STIM2 is a Ca^{2+} sensor inside the ER with low affinity for Ca^{2+}. This means that STIM2 should sense Ca^{2+} concentrations inside the ER when Ca^{2+} stores are filled at around >500 μM. A little decrease below this value should activate STIM2 and likely SOCE to keep the store filled. Consistently with this view, STIM2 knockout in normal cells decreases SOCE. In tumor cells, the loss of STIM2, leaves STIM1 in charge of sensing and refilling the stores. However, STIM1 is a different sensor with lower affinity for Ca^{2+} than STIM2 (Kd values around 300 μM). In this scenario, in which STIM1 would became activated only if stores are substantially depleted, the loss of STIM2 should lead to a partial depletion of Ca^{2+} stores (Fig. 19.2).

The above possibility was tested experimentally using siRNA against STIM2 in normal cells [69]. The decrease in STIM2 expression leads to the partial depletion of Ca^{2+} stores suggesting that STIM2 loss in tumor cells may contribute to Ca^{2+} remodeling by modifying Ca^{2+} store content. This partial depletion may have two relevant functional consequences. First, it may move Ca^{2+} store content close to the threshold for SOCE activation. Second, as Ca^{2+} store content has been related to the intrinsic pathway for cell death, loss of STIM2 in tumor cells may favor resistance to cell death and cell survival. Consistently, STIM2 silencing in normal cells decreases Ca^{2+} store content and increases resistance to apoptosis induced by H_2O_2 [69]. Therefore, the reciprocal shift in STIM1/STIM2 observed in colon cancer cells may be critical in colorectal tumorogenesis. Consistently with this view, it has been recently reported that STIM1 overexpression promotes colorectal cancer progression, cell motility and COX-2 expression [36].

In summary, colon cancer cells display enhanced and modified store and agonist-induced Ca^{2+} entry together with enhanced agonist-induced Ca^{2+} release and decreased Ca^{2+} store content. These differences are likely mediated by reciprocal

changes in the expression of ORAI1, STIM1, TRPC1 and STIM2. These changes contribute to increased cell proliferation, invasion and survival. Therefore, although the above results must be confirmed in samples from colorectal cancer patients, the data strongly suggest a critical role for changes in SOCE and SOCs in colon cancer tumorigenesis. In fact, with the logical limitations on the role of SOCE in the immune response against tumors, SOCE antagonists could be considered for colon cancer. Previous results are consistent with this view. For example, it has been extensively documented in in vitro assays, animal testing and even clinical trials with high risk patients that aspirin may efficiently prevent colon cancer. Interestingly, our previous results clearly showed that the main aspirin metabolite salicylate inhibits SOCE off site and colon cancer cell growth in a mitochondria-dependent manner suggesting that aspirin may prevent colon cancer acting on SOCE [70].

19.6 Aspirin Prevents Cancer

A large series of epidemiological evidences suggest that aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) prevent colorectal cancer and other forms of cancer including breast cancer [71]. A recent meta-analysis showed that NSAIDs decreased the frequency of adenomas, colorectal cancer and deaths related to colorectal cancer in 57 out of the 59 studies carried out between 1988 and 2006 [72]. Yet, the lack of clinical trials and the risk of secondary effects associated to chronic NSAID use prevented recommendation of aspirin. Recently, a few clinical trials have been completed in high risk patients including Lynch syndrome patients, a type familial colorectal cancer with 100 % chances of developing colorectal cancer. Aspirin protected largely (63 %) against cancer in these high risk patients which has resulted in recommendation of aspirin for high risk patients of colorectal cancer [73]. Ongoing clinical trials suggest that combinations of aspirin or other NSAIDs with additional chemopreventive compounds may be highly efficient in preventing polyp formation and cancer death in patients that had undergone surgery for tumor removal. These are high risk patients with a 50 % chance of recurrence and death. Similar trials are underway in other forms of familial cancer including breast cancer associated to driving mutations in BRCA1 and 2.

Basic studies carried out in cell lines and animal models of cancer indicate that aspirin and other NSAIDs inhibit tumor cell proliferation and growth, cell migration and invasion and tumor growth in animal models of cancer. Interest on the action mechanism is growing since the realization that a large part of the effects are largely independent of the anti-inflammatory activity of these drugs. This view is based in that antitumor activity remains in tumor cells lacking expression of COX, the classic target of anti-inflammatory compounds. Moreover, structural analogues like R-flurbiprofen that lack ant-inflammatory activity are also efficient in preventing tumor cell growth. Therefore, even though COX-mediated synthesis or prostanoids may contribute to inflammation and colon tumorogenesis and NSAIDs may act partially by preventing inflammation, other targets of aspirin and other NSAIDs

are likely involved in the antitumor actions of these drugs. We and others have shown previously that the aspirin metabolite and other NSAIDs may inhibit SOCs and SOCE in colon cancer cells likely providing a candidate mechanism for cancer chemoprevention by these compounds and a novel target for cancer chemoprevention.

19.7 Aspirin and Other NSAIDs Inhibit SOCE

NSAIDs inhibit SOCE and colon cancer cell proliferation [74]. However, no mechanism of action was provided. A few years ago, our group proposed that the main metabolite of aspirin, salicylate, could prevent tumor cell growth by inhibiting SOCE off site in a mitochondria-dependent manner [70, 75]. Salicylate is a mild mitochondrial uncoupler. This is due to the fact that the negative charge of the carboxylic residue is delocalized throughout the aromatic ring of salicylate. Thus, salicylic acid is neutral and capable of entering the cell and the mitochondrial matrix down a chemical gradient without restriction. However, once inside mitochondria, the matrix pH favors salicylic acid dissociation leading to salicylate formation that can exit the matrix favored by the negative mitochondrial potential. The net result is the release of protons inside mitochondria and mitochondrial uncoupling, partially depolarizing mitochondria and limiting the electromotive force for mitochondrial Ca^{2+} uptake [70]. Inasmuch as SOCE is strongly regulated by mitochondria, salicylate effect promotes the Ca^{2+} -dependent inactivation of Icrac and inhibition of SOCE. Since a sustained SOCE pathway is required for cell proliferation, the result is that salicylate inhibits cell proliferation via inactivation of a cancer-relevant Ca^{2+} channel. This mechanism may contribute to explain the antiproliferative effects of aspirin on T lymphocytes and vascular smooth muscle cells [70, 76, 77]. Interestingly, it has been recently reported that STIM1 overexpression promotes colorectal cancer progression, cell motility and COX-2 expression [36]. Accordingly, the effects of NSAIDs on SOCE may inhibit also colorectal cancer progression by preventing SOCE-mediated expression of COX-2.

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