Cell Calcium 81 (2019) 59–66

Contents lists available at ScienceDirect

Cell Calcium

journal homepage: www.elsevier.com/locate/ceca

TRPC1 and ORAI1 channels in colon cancer

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ARTICLE INFO

Keywords:
Colorectal cancer
TRPC1 channels
Store-operated Ca^{2+} entry
Store-operated currents
Mitochondria
Polyamines

ABSTRACT

Colon cancer cells, like other types of cancer cells, undergo the remodeling of the intracellular Ca^{2+} homeostasis that contributes to cancer cell hallmarks including enhanced cell proliferation, migration, and survival. Colon cancer cells display enhanced store-operated Ca^{2+} entry (SOCE) compared with their non-cancer counterparts. Colon cancer cells display an abnormal expression of SOCE molecular players including Orai1 and TRPC1 channels, and the stromal interacting molecule (STIM) 1 and 2. Interestingly, upregulation of Orai1 and TRPC1 channels and their contribution to SOCE are associated with cancer malignancy in colon cancer cells. In a specific cellular model of colon cancer, whereas in non-cancer colon cells SOCE is composed of the Ca^{2+} release activated (CRAC) currents, in colon cancer cells SOCE is composed of CRAC- and cationic, non-selective store operated (SOC) currents. Former SOCs are mediated by TRPC1 channels. Moreover, colon cancer cells also display dysregulation of the expression of 1,4,5-triphosphate receptors (IP_3R) that could contribute to the enhanced SOCE. Another important factor underlying the enhanced SOCE is the differential mitochondrial modulation of the CRAC and SOC currents in non-cancer and colon cancer cells. In colon cancer cells, mitochondria take up more Ca^{2+} that prevent the Ca^{2+}-dependent inactivation of the SOCs, leading to sustained Ca^{2+} entry. Notably, the inhibition of SOCE in cancer colon cells abolishes their cancer hallmarks. Robust evidence has shown the efficiency of non-steroidal anti-inflammatory drugs (NSAIDs) and difluoromethylornithine (DFMO) to reverse the enhanced cell proliferation, migration, and apoptosis resistance of cancer cells. In colon cancer cells, both NSAIDs and DFMO decrease SOCE, but they target different molecular components of SOCE. NSAIDs decrease the Ca^{2+} uptake by mitochondria, limiting their ability to prevent the Ca^{2+}-dependent inactivation of the SOCs that underlie SOCE. On the other hand, DFMO inhibits the expression of TRPC1 channels in colon cancer cells, eliminating their contribution to SOCE. The identification of players of SOCE in colon cancer cells may help to better understand the remodeling of the Ca^{2+} homeostasis in cancer. Importantly, the use of different pharmacological tools that target different SOCE molecular players in colon cancer cells may play a pivotal role in designing better chemoprevention strategies.

1. Adenomatous polyposis coli gene and polyamine biosynthesis in colorectal cancer

Colon cancer, and more generally, colorectal cancer (CRC), is one of the most prevalent forms of cancer with nearly 1.5 M new cases worldwide every year, half of them dying because of CRC in a few years [1]. CRC can be closely monitored by colonoscopy. In addition, the molecular history of CRC is well known. Therefore, this form of cancer has been used widely as a model for cancer chemoprevention. Nearly 80% of all CRC cases are related to alterations in the adenomatous polyposis coli (*APC*), the gene responsible for familial adenomatous polyposis. *APC* is a critical tumor suppressor and mutations in this gene are found in most colon cancers [2]. The APC protein binds β-catenin which is a key player in the Wnt pathway. The activation of Wnt receptor signals to APC and activates glycogen synthase kinase-3 (GSK-3) that phosphorylates β-catenin and mark it for proteosomal degradation [3]. In colon cancer cells, defective APC disturbs the Wnt pathway leading to cytoplasmic accumulation of β-catenin that translocates to the nucleus and binds the T-cell factor/lymphoid enhancer factor (TCF/LEF). The β-catenin-TCF/LEF complex activates the transduction of the KRas and Myc genes, which in turn promote the expression of other genes such as the ornithine decarboxylase (*ODC*) gene. The *ODC*

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https://doi.org/10.1016/j.ceca.2019.06.003
Received 30 March 2019; Received in revised form 24 May 2019; Accepted 6 June 2019
Available online 07 June 2019
0143-4160/

product is the rate-limiting enzyme involved in polyamine biosynthesis, particularly the catalysis of ornithine to putrescine [4]. Epithelia wounding induces the transient expression of the ODC enzyme and polyamine biosynthesis that act as local growth factors promoting proliferation and migration of epithelial cells for tissue repair [5].

It is well established that ODC is also upregulated in cancer cells, leading to a sustained increase in polyamine biosynthesis that contributes to cancer cell hallmarks including enhanced proliferation and cell migration. In support of this view, several studies indicate that inhibition of ODC with difluoromethylornithine (DFMO or eflornitine) decreases cancer hallmarks in skin cancer cells, bladder cancer cells, neuroblastoma, and colon cancer cells [4–6].

2. TRPC1 and Orai1 channels in store-operated Ca^{2+} entry (SOCE)

Ca^{2+} signaling is essential in several cellular functions including gene expression, cell proliferation, cell migration, differentiation, apoptosis, among others [7–9]. The levels of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) are finely tuned in space and time producing a number of different intracellular Ca^{2+} signals that regulate specific cellular functions. Intracellular Ca^{2+} signals comprise transient or sustained rises in $[Ca^{2+}]_{cyt}$, Ca^{2+} oscillations, Ca^{2+} rises restricted to subcellular regions (microdomains), or Ca^{2+} rises that spread throughout the entire cell [10,11]. In resting conditions, $[Ca^{2+}]_{cyt}$ is maintained in the nM range due to the operation of Ca^{2+} pumps and exchangers. For instance, the plasma membrane Ca^{2+} ATPase (PMCA) extrudes the Ca^{2+} from the cytosol to the extracellular space [12]. At intracellular level, Ca^{2+} is transported from the cytosol to the endoplasmic reticulum (ER) via the sarcoplasmic/endoplasmic-reticulum Ca^{2+} ATPase (SERCA) [13]. Moreover, mitochondria can take up Ca^{2+} via the mitochondrial Ca^{2+} uniporter (MCU) [14]. After cell stimulation, the $[Ca^{2+}]_{cyt}$ increases achieving levels in the μM range because of the Ca^{2+} influx via plasma membrane ion channels and/or the Ca^{2+} release from intracellular stores via 1,4,5-triphosphate receptors (IP_3R) and ryanodine receptors (RyR). This process may take milliseconds, as in muscle contraction and neurotransmitter release, or may last for longer periods of time participating in other cell functions as gene transcription, cell proliferation, and apoptosis. Alterations in the fine-tuning of Ca^{2+} signaling lead to dramatic changes in cell physiology. For instance, cancer cells show abnormal intracellular Ca^{2+} homeostasis and Ca^{2+} signaling that converge with cancer hallmarks [15–19].

In non-excitable cells, the store-operated Ca^{2+} entry (SOCE) is the major Ca^{2+} entry pathway, although this mechanism is also operational in electrically excitable cells. After cell stimulation with an agonist, or experimentally via the inhibition of the SERCA, the Ca^{2+} depletion of the ER activates SOCE. The transient receptor potential cation channels, particularly TRPC1, were originally proposed as the responsible for the store-operated current (SOC) that underlies SOCE [20,21]. However, subsequent evidence found that heterologous expression of TRP channels in Chinese ovary (CHO) cells and in the human embryonic kidney HEK293 cells do not contribute to SOCE in those cells [22,23]. On the other hand, it is widely accepted that the Ca^{2+} release-activated current (CRAC) [24] mediated by Orai1 channels [25] and gated by their interaction with the stromal interacting molecule 1 (STIM1) [26] is the main responsible for SOCE [27–30]. Even though this controversy, it has been found that the knockdown of endogenous TRPC1 decreases SOCE [31–34]. Moreover, in striatal medium spiny neurons, it has been demonstrated the involvement of TRPC1 in SOCE [35]. Although the lack of complete understanding of the role of TRPC1 in SOCE, there is growing evidence that in some cell types, TRPC1 in combination with Orai1 and STIM1 contributes to SOCE [36]. In human salivary gland cells, SOCE is composed of SOC and CRAC currents mediated by Orai1, TRPC1, and STIM1. In those cells, TRPC1 channels are recruited to the cell membrane by rises in $[Ca^{2+}]_{cyt}$ mediated by Orai1 [37]. More recently, it has been demonstrated that in Müller glial cells SOCE is mediated by Orai1 and TRPC channels [38]. Consistently, in rat β-cell, SOCE is mediated by channel complex of Orai1 and TRPC1 that are modulated by STIM1 [39]. Interestingly, it has been proposed that changes in the contribution of TRPC1 and Orai1 channels to SOCE are associated with pathologies such as cancer [19,40,41].

Another relevant issue is how physiological stimuli activate SOCE without compromising ER functions and delivering signals beyond the junctions. It has been reported that IP_3 receptors involved in physiological stimulation of SOCE are immobilized alongside the ER-PM junctions where SOCE takes place [42]. This sort of clustering provides efficient yet only local depletion of Ca^{2+} stores required to activate SOCE without influencing largely Ca^{2+} store level in ER areas located further. Accordingly, it has been proposed that each ER-PM junction may form a sort of autonomous SOCE unit driven by associated licensed IP_3Rs. This might operate at low stimulus that evoke Ca^{2+} oscillations requiring SOCE but no substantial Ca^{2+} store depletion [42]. Whether these units are altered in pathologies is not known yet.

3. SOCE, and Orai1 and TRPC1 channels in colon cancer

Remodeling of SOCE and their molecular players (Orai1-3 channels, TRP channel, and STIM1 and STIM2) has been associated with cancer hallmarks such as enhanced cell proliferation, migration and invasion, metastasis and apoptosis resistance, among others. Compared with their non-cancer counterparts, SOCE is enhanced in several types of cancer cells including the human esophageal squamous carcinoma cells [43], human breast cancer cells [44,45], glioblastoma multiform cells [46], human melanoma cells [47], and colon cancer cells [19]. Since the first demonstration that SOCE mediated by STIM1 and Ora1 in breast cancer cells is crucial for the enhanced cancer cell migration [45], growing evidence has shown the correlation between STIM1 and Orai1 upregulation and cancer hallmarks [18,48,49]. For instance, breast cancer cells, multiple myeloma cells, non-small cell lung cancer cells, human esophageal squamous carcinoma cells, and colon cancer cells display an enhanced SOCE associated with the upregulation of Orai1 channels. Notably, in those cells, the pharmacological inhibition of SOCE and SOCE players, as well as the knock down of Orai1 expression, decreases the cell proliferation, cell viability, and cell migration. Moreover, SOCE inhibition also arrests the cell cycle and induces apoptosis [19,41,43,50]. To address the remodeling of SOCE in colon cancer, two well-established models of human colon non-cancer (NCM460) cells [51] and HT29 cancer cells were analyzed in detail. Colon cancer cells display an enhanced SOCE compared with the non-cancer cells. Interestingly, the stimulation with the agonists ATP or carbachol activate SOCE in colon cancer cells but not in non-cancer colon cells. The remodeling of SOCE in colon cancer cells correlates with the upregulation of STIM1 and Orai1, as well as the appearance of TRPC1 channels as contributors to SOCE [19]. Subsequent evidence has corroborated that in cancer cells obtained from patients with CRC, STIM1 is upregulated, relative to their non-cancer counterparts. Additionally, the enhanced expression of both STIM1 and TRPC1 correlates with CRC aggressiveness [40,41].

The remodeling of SOCE in colon cancer cells correlates with the enhancement of the underlying SOCs as well as changes in the expression of the involved membrane ion channels. In non-cancer colon cells, SOCE is composed by Orai1-mediated CRAC currents. In contrast, in colon cancer cells, SOCE is composed of the combination of CRAC and cationic, non-selective SOCs. This former SOC currents are mediated by Orai1 and TRPC1 channels [19]. Further analysis revealed that SOCE and CRAC currents in non-cancer NCM460 cells are mediated by Orai1 channels interacting probably with STIM1 and STIM2 proteins. In contrast, SOCE and the SOCs in colon cancer HT29 cells involve Orai1, TRPC1 channels, and STIM1. A subsequent study has corroborated that enhanced SOCE in colon cancer cells is mediated by ion channels complex of TRPC1 and Orai1 [40]. In other cancer types, as in non-small cell lung cancer, the enhanced SOCE is also associated with the

upregulation of Orai1 and TRPC1 channels [41]. In addition, TRPC1 is involved in the enhanced migration and invasiveness of glioma cells [52] and thyroid cancer cells [53]. In the same line, a recent study has found that the inhibition of TRPC1, and other TRPC channels, decreases the aggressiveness of the transitional gastric cancer cells [54]. In agreement, recently it was demonstrated that TRPC1 channels are crucial to the cisplatin-induced apoptosis in non-small cell lung carcinoma cells [55]. Despite that in some of the described studies it was not examined whether the TRPC1 channel is contributing to SOCE, these channels have emerged as an important player in the remodeling of the intracellular Ca^{2+} homeostasis in diverse cancer cells. Additionally, the enhancement of SOCE observed in colon cancer cells can be explained by the changes in the expression of the other molecular players, i.e., Orai1 and STIM1. However, further studies are needed to understand the role of other components of SOCE. Importantly, it has been shown that other members of Orai, TRPC and STIM families are also dysregulated in cancer cells [15,48,56].

In addition to STIM1, Orai and TRPC1, the intracellular Ca^{2+} stores (ER, mitochondria, the Golgi apparatus, and the nucleus) are also remodeled in cancer cells. The ER is the main source of the Ca^{2+} that is released after cell stimulation and is crucial because its depletion activates SOCE. In non-excitable cells, the ER Ca^{2+} levels are mainly maintained by SERCA, IP_3Rs and buffering proteins like calreticulin. SERCA pumps import Ca^{2+} from the cytosol to the ER lumen [57] and IP_3Rs mediate the Ca^{2+} efflux from the ER lumen to the cytosol [58]. It has been demonstrated that cancer cells display either upregulation or downregulation of the SERCA and the IP_3Rs [59]. For example, relative to non-cancer cells, the SERCA isoform 2 is upregulated in colon cancer cells, and this change correlates with cancer malignancy [60,61]. Conversely, in colon adenomas, the expression of the SERCA isoform 3 is decreased relative to non-cancer colon cells [62,63]. On the other hand, the release of Ca^{2+} from ER is mediated by IP_3Rs, which have three known isoforms (IP_3R1-3) [58]. The IP_3R2 and IP_3R3 are upregulated in lymphoma [64] and glioma cells [65], respectively. Moreover, IP_3R1 and IP_3R2 are expressed in both non-cancer and cancer colon cells, but the IP_3R3 is exclusively found in cancer cells and its expression correlates with cell invasiveness and metastasis [66]. Although evidence indicates that cancer cells display an abnormal expression of the SERCA and the IP_3Rs, the consequences in the ER Ca^{2+} content and SOCE are largely unknown. In colon cancer cells (HT29) [19] and prostate cancer cells (LNCaP), the ER Ca^{2+} content is decreased relative to their non-cancer counterparts. In LNCaP, the decrease in ER Ca^{2+} content is due to an increase of the Ca^{2+} leak from the ER due to the upregulation of the IP_3R1 and the increase of its phosphorylation [67]. It has been suggested that ER Ca^{2+} content is associated with Ca^{2+}-induced apoptosis. The decrease of the ER Ca^{2+} content might be responsible of the resistance to the H_2O_2-induced apoptosis in colon cancer cells [19]. Then, besides its role in the activation of SOCE, ER might play other important roles in different cancer processes. Despite these data indicate that dysregulation of the expression of IP_3Rs correlates with cancer hallmarks, further studies are needed to understand the participation of all the IP_3R isoforms, the SERCA pumps, and the ER Ca^{2+} content in SOCE remodeling in cancer cells and their roles in cancer hallmarks. In summary, colon cancer cells show an enhanced SOCE that is associated with changes in the expression of the molecular players involved including the membrane ion channels (Orai1 and TRPC1), the ER Ca^{2+} sensors (STIM1 and STIM2), the SERCA pumps, and the IP_3Rs. Fig. 1 illustrates the major findings of the remodeling of those molecular players of SOCE in cancer colon cells.

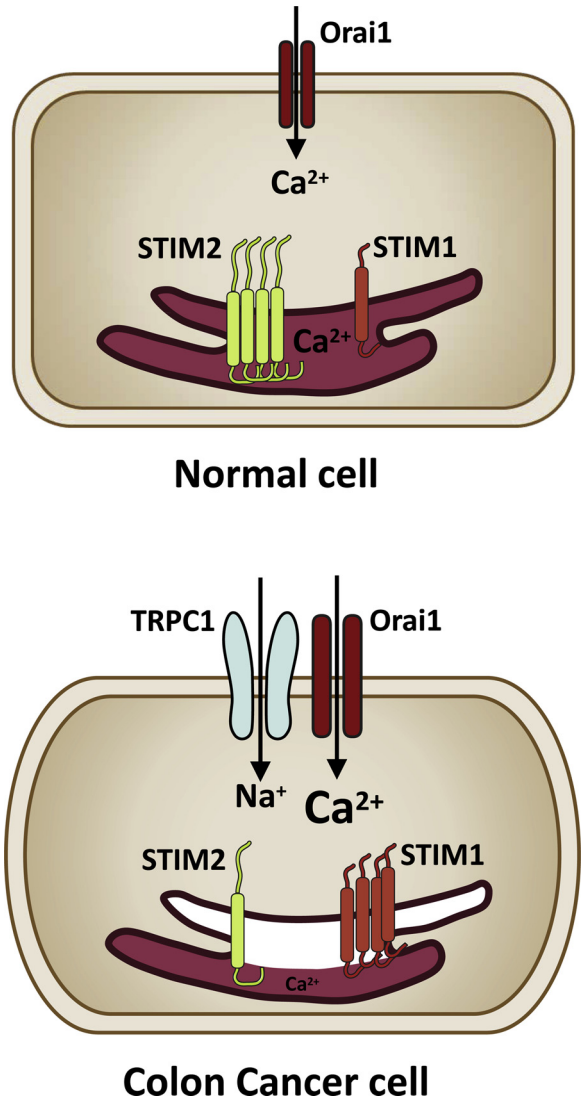

Fig. 1. SOC remodeling in colon cancer cells.
In normal mucosa cells, SOCE is small and Ca^{2+} stores at the endoplasmic reticulum are full. SOCE is mediated by Orai1 channels gated by both STIM1 and STIM2 proteins at the endomembranes. In colon cancer cells, SOCE is much larger and Ca^{2+} stores are partially empty. SOCE is mediated by TRPC1 and Orai1 channels gated only by STIM1.

4. Mitochondria control of SOCE and SOCs in colon cancer cells

As stated above, SOCE is activated after release of Ca^{2+} from intracellular stores that promotes STIM1 oligomerization and its interaction with Orai1 in the plasma membrane. However, activation and inactivation of CRAC channels is not solely dependent on the filling state of Ca^{2+} stores. It is well known that CRAC channels inactivate in a Ca^{2+} dependent manner, thus providing a feedback regulatory mechanism for avoiding sustained Ca^{2+} entry [68,69]. Accordingly, endogenous Ca^{2+} buffers are important for modulating SOCE and CRAC. One of the major endogenous Ca^{2+} buffers are mitochondria. Mitochondria are able to take up Ca^{2+} by the mitochondrial Ca^{2+} uniporter (MCU), a Ca^{2+}-activated Ca^{2+} channel located at the inner mitochondrial membrane [70]. The mitochondrial membrane potential, created by H^+ exit through the mitochondrial complexes, provides the driving force for mitochondrial Ca^{2+} uptake. However, mitochondria are not Ca^{2+} stores. Thus, once Ca^{2+} enters mitochondria, it may return to the cytosol in exchange for Na^+ by the mitochondrial Na^+/Ca^{2+} exchanger (NCLX) [71]. In resting conditions, no much Ca^{2+} enters mitochondria because MCU is closed unless surrounding Ca^{2+} reaches high values above 5 to 10 μM [72]. After cell activation, mitochondria may take up large amounts of Ca^{2+} and mitochondrial $[Ca^{2+}]$ may reach transiently even the mM level, provided that mitochondria are close enough to Ca^{2+} channels in the plasma membrane

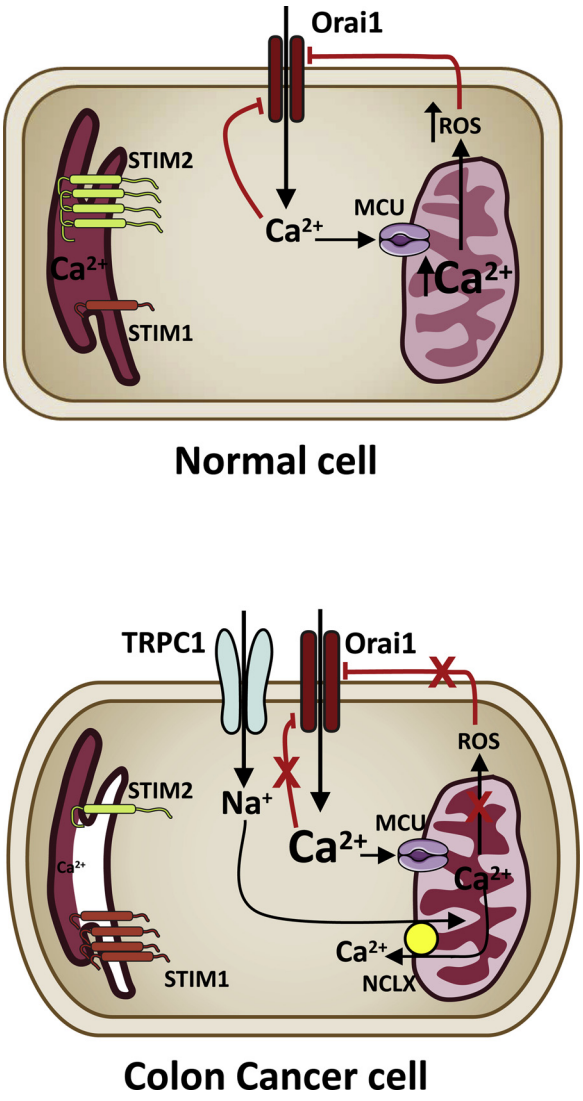

Fig. 2. Mitochondrial control of SOCs in colon cancer cells.
In normal mucosa cells, SOCE is mediated by Orai1 channels. Orai1 channels inactivate in a Ca^{2+} dependent manner and mitochondrial Ca^{2+} uptake promotes generation of reactive oxygen species (ROS) that promote further Orai1 channel inactivation. In colon cancer cells, the large mitochondrial Ca^{2+} uptake driven by enhanced mitochondrial potential prevents the Ca^{2+} dependent inactivation of Orai1 channels. In addition, Na^{+} influx mediated by TRPC1 channels favors Ca^{2+} exit from mitochondria that limits ROS dependent inactivation of Orai1 channels. Thus, mitochondria and TRPC1 channels contribute to sustain SOCs in colon cancer cells.

or the ER [73].

In some cell types, the ability of mitochondria to take up Ca^{2+} is essential for modulating Ca^{2+} entry through SOCE. For instance in T cells, mitochondrial Ca^{2+} uptake is essential to avoid the Ca^{2+} dependent inactivation of CRAC channels [68,69]. This is also the case in colon cancer cells where mitochondrial uncoupling decreases SOCE leading to inhibition of cell proliferation [74,75]. One of the characteristics of tumor cells is the Warburg effect by which tumor cells use glycolisis instead mitochondrial respiration even in the presence of oxygen. This process has growth advantages for survival despite that energy production might be less efficient. One of the consequences of the Warburg effect is the increased mitochondrial potential that influences mitochondrial Ca^{2+} homeostasis. The consequences of the Warburg effect for mitochondrial control of SOCE have been recently addressed [76]. Specifically, mitochondrial control of SOCE in normal and colon cancer cells has been recently investigated [76]. CRAC currents in normal physiological conditions inactivate in a Ca^{2+} dependent manner since inactivation is counteracted in strong intracellular Ca^{2+} buffer (Fig. 2). Moreover, addition of a mitochondrial cocktail (intented to increase mitochondrial respiration and potential), enhances CRAC activation but fails to prevent CRAC inactivation in non-cancer colon cells [76]. In colon cancer cells, the TRPC1-dependent component of store-operated currents is not affected by intracellular Ca^{2+} buffering. However, the Orai1-dependent component displays strong Ca^{2+}-dependent inactivation. This inactivation is removed in the presence of strong intracellular Ca^{2+} buffering [76]. In contrast to normal cells, in colon cancer cells, the mitochondrial cocktail reverses the inactivation of the current. In other words, mitochondria modulate activation of CRAC in normal cells but these organelles are not able to prevent current inactivation. However, in colon cancer cells mitochondria prevents current inactivation, thus sustaining the current (Fig. 2). Consistently, mitochondria depolarization with uncouplers promotes the current inactivation unless intracellular Ca^{2+} is strongly buffered [76]. Accordingly, mitochondria modulate differentially SOCs and SOCE in normal colonic and colon cancer cells.

As stated above, in non-cancer colon cells, mitochondria are unable to prevent CRAC inactivation. Nevertheless, they are required for SOCE operation since without mitochondria CRAC do not activate or activate minimally. This is against the prevalent view that mitochondria control inactivation of CRAC in T cells [77]. In this case, mitochondrial Ca^{2+} uptake may be highly efficient because of the strategic location of mitochondria close to the immunological synapse, this making mitochondria to sense efficiently Ca^{2+} microdomains formed underneath the immunological synapsis for quick and massive mitochondrial Ca^{2+} uptake, thus preventing CRAC inactivation for sustained Ca^{2+} signaling [77]. This may not apply to non-cancer colon cells. However, in colon cancer cells, the ability of mitochondria to take up Ca^{2+} may be enhanced. One mechanism for this change could be a closer localization of mitochondria to Orai1 channels in colon cancer cells. However, evidence for this possibility is lacking. The other possibility is that mitochondrial Ca^{2+} uptake increases in colon cancer cells because of the Warburg effect and the related increase in mitochondrial potential [78]. Consistently, mitochondrial potential and mitochondrial Ca^{2+} uptake are larger in colon cancer cells than in non-cancer colon cells [76]. In addition, transcriptional analysis of genes involved in mitochondrial Ca^{2+} transport indicate that genes modulating positively the mitochondrial Ca^{2+} uniporter are overexpressed in colon cancer cells while negative modulators are downregulated [79].

There is still another factor that may explain differential SOCE modulation by mitochondria in normal and colon cancer cells: the different nature of CRAC and SOC. On one hand, CRAC channels in colon cancer cells are upregulated, thus leading to enhanced currents that introduce more Ca^{2+} that may promote more efficient mitochondrial Ca^{2+} uptake in tumor cells than in normal cells. On the other hand, the recruitment of non-selective TRPC1 channels permeable to Na^{+} in colon cancer cells but not in the normal cells. It has been recently reported that Na^{+} permeability may be essential for mitochondrial control of CRAC inactivation. Specifically, it has been shown that SOCE activates a mitochondrial redox transient which is dependent on the mitochondrial Na^{+}/Ca^{2+} exchanger (NCLX) that is required for preventing Orai1 inactivation through oxidation of a critical cysteine (Cys195) in the third transmembrane helix of Orai1 [80]. This mechanism remains controversial and has been recently challenged in RBL cells [81]. However, If Orai1 channels are indeed regulated by ROS, it is possible that in non-cancer colon cells, lacking TRPC1 channels and Na^{+} influx, NCLX could be prevented, thus leading to ROS generation and oxidation of Orai1 at Cys195 and the ensuing channel inactivation (Fig. 2). In colon cancer cells, the presence of TRPC1 channels and store-operated Na^{+} influx, enables Ca^{2+} exit from mitochondria through NCLX, thus preventing ROS, ROS-dependent oxidation of Cys195 and Orai1 inactivation, thus sustaining SOCE [82] (Fig. 2). This possibility warrants further consideration.

Regardless of the mechanisms involved, SOCs in colon cancer cells are sustained by the mitochondrial ability to take up Ca^{2+} and prevent the Ca^{2+}-dependent inactivation of these channels [76]. Accordingly, mitochondria could be considered novel targets to promote CRAC inactivation in cancer cells. Consistently, aspirin metabolite salicylate and other NSAIDs that are mild mitochondrial uncouplers inhibit mitochondrial Ca^{2+} uptake [74–76,83,84]. As a consequence, NSAIDs promote the Ca^{2+}-dependent inactivation of CRAC, thus preventing SOCE and cell proliferation in different cell types including colon cancer cells [74–76], rat basophilic leukemia (RBL) cells [83] and vascular smooth muscle cells (VSMC) [83,84]. These effects are entirely dependent on Ca^{2+} influx because they are reversed simply by enhancing extracellular Ca^{2+} concentration [74]. In addition, CRAC inactivation by NSAIDs is prevented in strong intracellular Ca^{2+} buffering, thus providing compelling evidence that NSAIDs do not inhibit CRAC channels directly. Instead, NSAIDs promote their Ca^{2+}-dependent inactivation [76]. Interestingly, NSAIDs inhibit fully CRAC and SOCE in non-cancer colon cells and vascular smooth muscle cells but only partially SOCE in colon cancer cells [76,82]. In fact, in colon cancer cells, NSAIDs promote the Ca^{2+} inactivation of Orai1 mediated currents but have no effect on the TRPC1 component of the current [74,76]. Given the pleiotropic role of SOCE in different cell types, and particularly the role of this pathway in cell proliferation and other cancer hallmarks, it is worthy of mention that the antiproliferating effects of NSAIDs in VMSCs and cancer cells could be mediated by Ca^{2+}-dependent inactivation of SOC channels. In addition, this mechanism could contribute to cancer chemoprevention elicited by aspirin and other NSAIDs.

5. Mitochondria and modulation of SOCE by reactive oxygen species in cancer cells

Mitochondria have been largely recognized as the main source of cellular energy throughout the synthesis of adenosine triphosphate (ATP) *via* the respiratory chain. The mitochondrial respiratory chain and the tricarboxylic acid cycle enzyme α-ketoglutarate dehydrogenase generate reactive oxygen species (ROS) as the superoxide ($O2\cdot^-$). Following, $O2\cdot^-$ could be converted to hydrogen peroxide (H_2O_2) and to hydroxyl radical (·HO) [85–87]. Although there are other sources of intracellular ROS, mitochondria are the major source of those highly reactive compounds [88,89]. As described above, in colon cancer cells the disturbance of mitochondria functions support cancer processes by preventing the Ca^{2+}-dependent inactivation of SOCE. It has been proposed that mitochondria also contribute to cancer processes through the production of ROS. In cancer cells, the levels of ROS are increased compared with non-cancer cells [90,91]. As recently reviewed in reference [48], the increase of intracellular ROS levels may support carcinogenesis by diverse mechanisms. However, it is largely controversial whether SOCE is enhanced or inhibited by the increased levels of ROS [92]. For instance, H_2O_2 inhibits Orai1 and Orai2 channels but does not affect Orai3 channels [93]. Moreover, if Orai1 channels are in complex with STIM1, as during the CRAC activation, H_2O_2 is unable to inhibit those channels. Apparently, the effects of H_2O_2 on Orai channels are mediated by the oxidation of specific cysteine residues that are present in Orai1 and Orai2, but not in Orai3 [94]. Similarly, ROS can modulate the activity of STIM1 *via* the oxidation of a cysteine residue in its luminal Ca^{2+}-binding domain. The ROS-induced oxidation of STIM1 lowers its Ca^{2+} affinity and promotes its oligomerization, producing store-independent Ca^{2+} entry [95]. These results indicate that ROS can modulate SOCE by redox reactions with the cysteine residues in STIM1 and Orai1 and Orai2 channels.

TRPC1 channels are also modulated by ROS. In pulmonary arterial smooth muscle cells, increments of ROS levels enhance SOCE and cell proliferation. Remarkably, in those cells the elevated ROS levels are accompanied by the upregulation of the TRPC1 and TRPC6 channels [96]. Currently, there is no data regarding the role of ROS in modulating SOCE in colon cancer cells. The evidence from the studies described above indicates that ROS inhibit the Orai1-dependent SOCE, but increases the TRPC1-dependent SOCE. Then, further studies are needed to understand the relationship between ROS and SOCE as well as their and impact in the colon cancer hallmarks.

6. Reversal of SOC remodeling in cancer by polyamine synthesis inhibition

As discussed above, SOCs in colon cancer cells differ from those in non-cancer colon cells in several characteristics: First, the plasma membrane channels involved (TRPC1 and Orai1 channels in colon cancer cells vs only Orai1 channels in normal colonic cells). Second, the number of Orai1 channels engaged (larger in colon cancer cells than in normal cells). Third, the gating of SOCs by STIM proteins (Only STIM1 in cancer cells vs both STIM1 and STIM2 in non-cancer cells). Fourth, the ability of mitochondria to prevent Ca^{2+}-dependent inactivation (weak in normal cells and strong in cancer cells). In addition, SOCE in cancer cells is prone to be activated because Ca^{2+} stores are partially depleted while in normal cells, Ca^{2+} stores are filled and SOCE activation in physiological conditions is hard to achieve. This remodeling of intracellular Ca^{2+} homeostasis partially resembles changes that occur during early mucosal restitution after wounding. In this scenario, resting epithelial cells are transiently transformed into proliferating and migrating cells that repair the wound and return to a quiescent state [97,98]. It has been shown that STIM1 translocation to the plasma membrane enhances intestinal epithelial restitution by inducing TRPC1-mediated Ca^{2+} signaling after wounding [97,98]. In addition, this pathway involving TRPC1-mediated Ca^{2+} signaling is mediated by differential modulation of STIM1 and STIM2 elicited by transient biosynthesis of polyamines [98].

Evidence indicate that excess polyamine biosynthesis is involved in colon carcinogenesis. In fact, the polyamine synthesis pathway has been linked to colon cancer. As stated above, APC, tumour suppressor gene that is either mutated or lacking in familial adenomatous polyposis (FAP) patients and samples of CRC, is able to modulate the expression of a number of genes, including the c-MYC oncogene. In turn, MYC regulates other genes among them those involved in the polyamine pathway, particularly ODC [4]. DFMO and NSAIDs reduce either polyamine synthesis and/or increase their export [5,6]. Moreover, levels of polyamines are enhanced in colorectal carcinoma cells compared to nonmalignant counterparts and are considered essential for neoplastic transformation in vitro [99]. Expression of ODC that converts ornithine to putrescine, the first polyamine, is dramatically enhanced in neoplastic colorectal tissue compared to adjacent normal tissue. Consistently, polyamines concentrations in excreted urine of CRC patients are enhanced and they tend to normalize after successful treatment. Finally, inhibition of polyamine biosynthesis virtually abolishes tumor growth unless polyamines are exogenously provided. Therefore, compelling evidence suggest the role of polyamines in CRC and that polyamine synthesis inhibition limits cancer growth. However, the mechanisms involved are barely known. Increasing evidence suggest polyamines act by stimulating proto-oncogene expression [99]. DFMO, the suicide inhibitor of ODC, prevents epithelial restitution and this effect involves differential modulation of STIM1 and STIM2.

The mechanisms of cancer chemoprevention by DFMO are not well established. However, studies in diverse types of cancer have demonstrated that DFMO combined with other chemicals as N1-guanyl-1,7-diaminoheptane or non-steroidal anti-inflammatory drugs (NSAIDs) reduces cancer hallmarks. The effects of DFMO on cancer cell hallmarks and Ca^{2+} remodeling has been addressed recently [100]. DFMO treatment inhibits colon cancer cell proliferation and decreases resistance to cell death [100]. In addition, DFMO treatment decreases SOCE and enhances Ca^{2+} store content in colon cancer cells, thus reversing Ca^{2+} remodeling in colon cancer cells and providing a basis for effects on proliferation and resistance to cell death [100]. Interestingly,

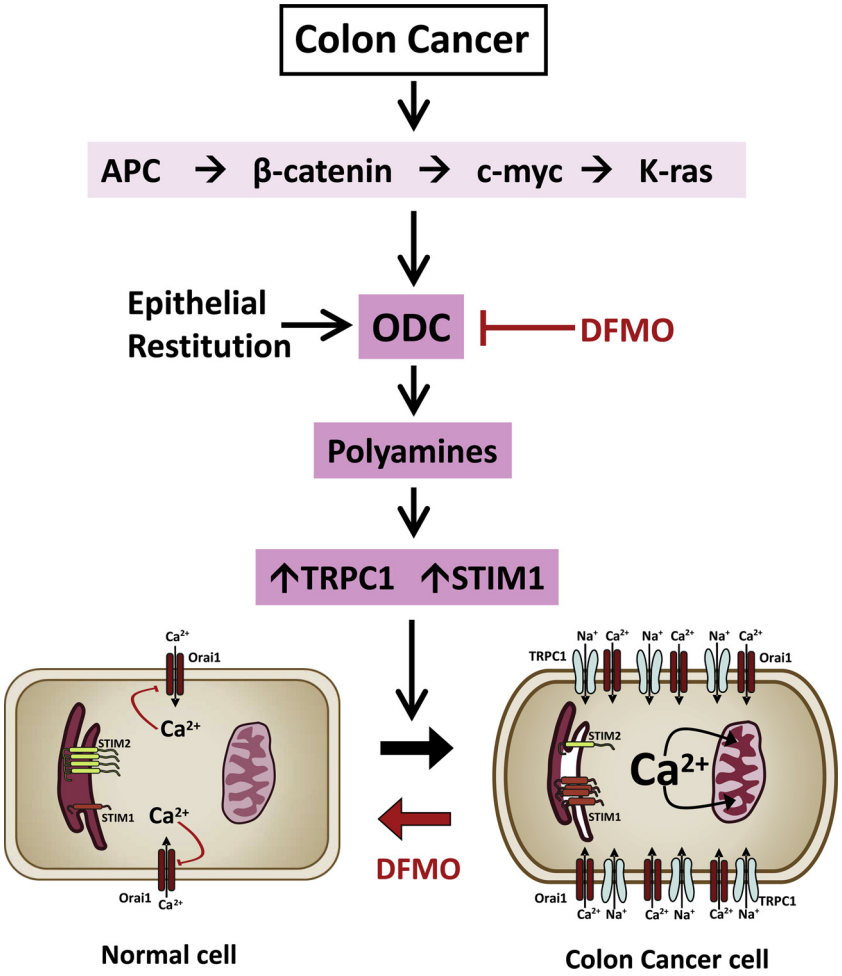

Fig. 3. Polyamines promote SOC remodeling in colon cancer cells.
In colon cancer, APC mutations allows β-catenin mediated c-myc and K-ras activation leading to overexpression of ornithinedecarboxylase (ODC) and polyamine biosynthesis. In turn, polyamines increase TRPC1 and STIM1 expression leading to SOC remodeling and sustained Ca^{2+} signaling. These effects are prevented and/or reversed by ODC inhibitor DFMO.

DFMO treatment removes specifically the TRPC1-dependent component of SOC, without affecting the inward component mediated mostly by Orai1. In addition, the effects of DFMO treatment are transient and reversed by polyamine putrescine [100]. Therefore, the effect of DFMO on TRPC1 channels depend on inhibition of polyamine synthesis. Consistently with these data, treatment of colon cancer cells with DFMO downregulated TRPC1 and STIM1 without affecting STIM2. Taken together, these data indicate that DFMO reverses SOC remodeling in colon cancer cells by inhibiting ODC. These data suggest then that ODC overexpression, one of the transcriptional targets of c-myc, and excess polyamine biosynthesis may contribute to SOC remodeling in CRC and the underlying cancer hallmarks (Fig. 3).

There is presently a renewed interest in DFMO for cancer chemoprevention. Emerging clinical trials provide strong evidence that DFMO, and combinations of DFMO with NSAIDs, specially sulindac, are particularly efficient in preventing CRC in individuals at high risk of developing CRC including patients whit surgically removed colorectal tumors [101,102]. Several NSAIDs including sulindac are able to prevent mitochondrial Ca^{2+} uptake and promote Ca^{2+}-dependent inactivation of CRAC, the Orai1 component of SOCs [76]. Interestingly, the effects of the combination of DFMO and sulindac on SOCs have been recently tested in colon cancer cells [100]. As stated above, DFMO abolishes selectively the TRPC1 component of SOCs whereas sulindac removes the Orai1 component. Consistently, combination of both DFMO and sulindac abolish both components and prevents fully SOCE [100]. Interestingly, the combination of DFMO and sulindac decreases efficiently CRC and is presently the basis of a large III phase clinical trial, the PACES trial [103]. These data suggest a critical role for SOC channel remodeling in colon cancer and that targeting this remodeling by DFMO/sulindac may be critical for colon cancer chemoprevention.

In summary, colon cancer cells undergo a deep remodeling of store-operated channels from small, transient and inactivating CRAC channels typical of non-cancer colon cells to large, non-selective and sustained currents characteristic of colon cancer cells. We propose the hypothesis that this remodeling can be largely explained by changes in expression of TRPC1 and STIM1 mediated by polyamine synthesis due to ODC overexpression secondary to APC deactivation and c-myc activation in CRC. This remodeling can be reversed, at least partially and/or transiently by ODC inhibition with DFMO in combination with NSAIDs, thus suggesting a critical role for SOCE remodeling in cancer chemoprevention.

Acknowledgements

We gratefully acknowledge support from the Spanish Ministry of Economy and Competitivity (grant BFU2015-70131R), Junta de Castilla y León, Spain (grant VA294P18), the Spanish Association Against Cancer (AECC), and a predoctoral fellowship of the University of Valladolid to LGG.

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