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Roles of CRAC channel in cancer: implications for therapeutic development

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Abstract

Introduction: The Ca2+release-activated Ca2+ (CRAC) channel, composed of Orai and STIM proteins, represents one of the main routes of Ca^{2+} entry in most non-excitable cells. There is accumulating evidence to suggest that CRAC channel can influence various processes associated with tumorigenesis. Overexpression of CRAC channel proteins has been observed in several types of cancer tissues and cells, indicating that blocking CRAC channel activated Ca^{2+} influx can have therapeutic benefits for cancer patients.

Areas covered: In this review, we have primarily focused on the molecular composition and activation mechanism of CRAC channel as well as the myriad roles this Ca^{2+} channel play in various cancers. We further describe relevant information about several efforts aimed at developing CRAC channel blockers and their likely implications for cancer therapy. We have extensively utilized the available literature on PubMed to this end.

Expert opinion: The possibility of targeting CRAC channel mediated Ca^{2+} entry in cancer cells has generated considerable interest in recent years. Use of CRAC channel blockers in cancer preclinical studies and clinical trials has been relatively limited as compared to other diseases. The future lies in developing and testing more potent and selective drugs that target cancer cell specific CRAC channel proteins, hence opening better avenues for cancer therapeutic development.

Keywords

Calcium; Cancer therapeutics; CRAC channel; CRAC channel inhibitor; Orai1; STIM1; Store operated Ca^{2+} entry

1. Introduction

As one of the most multifaceted, ubiquitous and dynamic intracellular secondary messengers, calcium (Ca^{2+}) is known to be involved in regulating multiple cellular processes, including cell proliferation, differentiation, apoptosis, migration, as well as cell

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contraction and secretion [1,2]. It is believed that dysregulation of Ca^{2+} cellular homeostasis could result in several pathological conditions such as cardiovascular diseases, neurodegenerative ailments, developmental abnormalities, diabetes, immunodeficiency disorders and cancer [3,4]. Maintaining the cellular homeostasis of Ca^{2+} depends on the synchronized functioning of various calcium channels, exchangers and pumps, which are located either on the plasma membrane of the cell or the membranes of endoplasmic reticulum and mitochondria [5,6].

One of the most important mechanisms of Ca^{2+} entry into the mammalian cell is known as the store operated Ca^{2+} entry (SOCE). SOCE, which results in an increase in the cytoplasmic Ca^{2+} concentration, is triggered by the release of Ca^{2+} stored in endoplasmic reticulum (intracellular Ca^{2+} store) and the subsequent entry of extracellular Ca^{2+} across the cell membrane through Ca^{2+} channels, that are referred to as calcium release activated calcium (CRAC) channels [7–9]. Ca^{2+} influx through CRAC channels causes the activation of various downstream signaling pathways which are involved in the regulation of gene expression, cell proliferation, apoptosis, cytokine generation and many other cellular activities [10]. CRAC channels are present widely across various cell types [11–16]. They possess extremely low conductance in comparison to other Ca^{2+} channels, while being highly selective for Ca^{2+} [17]. Several studies have linked altered CRAC channel activity with various ailments, such as thrombosis, pancreatitis, inflammatory bowel disease, severe combined immunodeficiency disorder, as well as cancer [18–23].

Cancer is one of the major causes of mortalities across the globe [24] and therefore, there is an ever-increasing need for identifying novel cellular and molecular components that can be targeted for effective therapy. In this regard, Ca^{2+} activated downstream signal transduction pathways have been considered promising cancer therapeutic targets [1]. Cancer cells, unlike their non-cancerous counterparts, are known to undergo continual Ca^{2+} signal remodeling due to changes in the activity and expression of calcium pumps and channels [25], as also alterations in the Ca^{2+} associated intracellular signaling pathways. It is believed that such a strategy is adopted by cancer cells in order to sustain their rapid growth and to circumvent cell death mechanisms [26]. Therefore, a clear understanding of Ca^{2+} signal remodeling and alterations in calcium channel protein expression in cancer would set the scene for development of new therapeutic drugs targeting cancer cells.

In this article, we review the relevant literature on the molecular identities of CRAC channel in context of the roles they play in cancer development. We also provide a critical overview of the therapeutic potential of various CRAC channel inhibitors for cancer treatment. Although numerous CRAC channel inhibitors have been developed over the years and their effects subsequently investigated on various humans diseases, we have limited ourselves to discussing only those inhibitors that have been reported to show some promising results on cancer models in preclinical studies or those that have reached clinical trials on cancer patients.

2. CRAC channel: molecular basis and mode of activation

CRAC channel comprises of a combination of two protein components - Orai and STIM. Orai, which is a tetra-spanning transmembrane protein, is situated on the cell membrane and is responsible for forming the ion channel pore through which extracellular Ca^{2+} enters the cell. It has three homologues, namely Orai1, Orai2 and Orai3. On the other hand, STIM (stromal interaction molecule) protein, which functions both as a sensor of endoplasmic reticulum Ca^{2+} levels as well as an activator of SOCE, is a single-pass transmembrane protein located on the membrane of endoplasmic reticulum. STIM is known to have two isoforms, STIM1 and STIM2. The combination of Orai1 and STIM1 is the prototypical, best characterized CRAC channel and is present in most of the mammalian cells [10,23].

Despite sharing significant structural homology, STIM1 and STIM2 exhibit remarkable differences in their properties and functionalities. Unlike STIM1, STIM2 can be activated by minor alterations in Ca^{2+} levels owing to a lower affinity towards Ca^{2+} [27]. Therefore, STIM1 is considered the primary sensor of Ca^{2+} concentration in the lumen of endoplasmic reticulum which enhances CRAC channel activity as a result of fast and considerable depletion of intracellular Ca^{2+} stores. In contrast, STIM2 stabilizes the level of Ca^{2+} following a slow and moderate depletion of Ca^{2+} stores and thereby acts as a housekeeping Ca^{2+} sensor in the endoplasmic reticulum [28,29]. Also, STIM1 is reportedly a more efficient activator of CRAC channel activity, possibly due to its rapid aggregation kinetics and stronger ability to associate with Orai [30,31]. In fact, all the three types of Orai proteins can complex with STIM1 to form functional CRAC channels. However, there is variation in their tissue distribution and Ca^{2+} selectivity [32]. It has also been reported that while Orai2 and Orai3 can be activated by the depletion of Ca^{2+} stores, the CRAC currents induced by them are weaker than those induced by Orai1 [33].

Ca^{2+} signaling in the cells gets activated either by the release of Ca^{2+} stored in the endoplasmic reticulum or the influx of extracellular Ca^{2+} into the cells. CRAC channel functions to combine and coordinate these two routes through which intracellular Ca^{2+} signaling may be induced. Figure 1 shows the mechanism of their activation under physiological conditions. The first step in the activation of CRAC channel involves the stimulation of cell surface receptors, such as G-protein coupled receptors (GPCRs) or receptors tyrosine kinases (RTKs), through binding of their respective ligands (agonists), that activates membrane-bound enzyme phospholipase C (PLC), which in turn hydrolyzes membrane phospholipid phosphatidylinositol-4,5-bisphosphate (PIP2) into inositol triphosphate (IP3) and diacylglycerol (DAG). The subsequent binding of IP3 to the Ca^{2+} permeable IP3 receptors located on the endoplasmic reticulum membrane elicits the release of Ca^{2+} from the endoplasmic reticulum into the cytosol [34,23]. This results in the depletion of Ca^{2+} levels in the lumen of endoplasmic reticulum, which is eventually sensed by STIM proteins. Consequently, STIM proteins respond by oligomerizing and translocating to the junction of endoplasmic reticulum and plasma membrane where they form large clusters, termed as STIM puncta. A cytoplasmic CRAC activation domain (CAD) on the C terminus of STIM directly interacts with the intracellular C terminus of Orai [35, 36]. This physical coupling of STIM and Orai proteins induces the opening of CRAC channel, thereby allowing the influx of extracellular Ca^{2+} into the cytosol. Ultimately, such an influx of Ca^{2+}

facilitates the replenishment of the depleted intracellular Ca^{2+} stores, in addition to evoking the activation of the Ca^{2+} associated signal transduction pathways.

Activation of CRAC channel, as described above, brings about a sustained increase in cytosolic Ca^{2+} levels that result in the activation of calmodulin (CaM), an intracellular Ca^{2+} sensor protein. Calmodulin, in turn, activates calcineurin, a Ca^{2+}/CaM dependent phosphatase. Calcineurin further causes the dephosphorylation of several phosphoserines located in the regulatory domain of NFAT (nuclear factor of activated T cells). This results in the translocation of NFAT into the nucleus, where it collaborates with various transcription factors to initiate the expression of several genes that are involved in the regulation of multiple cellular processes such as proliferation, survival, migration and angiogenesis [37]. Ca^{2+} entry through CRAC channels is also known to activate other transcription factors like NF-kB (nuclear factor-kB) and CREB (cAMP responsive element binding protein). NF-kB is activated through activation of IKK (IkB kinase), whereas CREB gets activated via CaMKII/IV (calmodulin dependent protein kinase II/IV) activation. NF-kB plays a critical role in different cellular phenomena including, carcinogenesis and inflammation [38]. Ca^{2+} mediated activation of CREB has been reported to enhance the proliferation of vascular smooth muscle cells [39].

3. CRAC channel and cancer

SOCE mediated through the activation of CRAC channels is considered to be the major route of Ca^{2+} entry in non-excitable cells [40,41]. Interestingly, elevated intracellular Ca^{2+} levels have been reported in various human and animal cancer cells [42], suggesting the possibility of an augmented Ca^{2+} entry through CRAC channels in malignant cells. Also, it is now well established that the Ca^{2+} influx mediated by CRAC channels and its ensuing signal transduction play quite significant and diverse roles in various cellular events associated with cancer development. These include regulation of cell cycle, cell proliferation, cell death, cell migration, invasion and metastasis. In addition, it also regulates tumor neovascularization and antitumor immunity [43].

A concise summary of the aberrant expression of CRAC channel proteins and their consequential effects as reported by several studies on different types of cancers is given in Table 1. Evidence from a considerable body of literature about the role of CRAC channel proteins in regulating some key cancer hallmarks for various types of cancers are enumerated below.

3.1 Breast cancer

Breast cancer cells remodel the expression and functional role of the molecular components of CRAC channel [44]. A microarray data analysis has shown that the breast cancer subtype with the poorest prognosis is linked to high STIM1 and low STIM2 tumor expression profile, indicating that alteration in CRAC channel pathway may be associated with poor prognosis in breast cancer patients [45]. A recent study has reported pro-survival and anti-apoptotic effects of Orai1 in T47D and MCF-7 breast cancer cells grown on a collagen coated surface [46]. The essential role of Orai1 and STIM1 in breast cancer cell migration and metastasis has been delineated by Yang et al. [22]. Silencing STIM1 or ORAI1 in highly

metastatic human breast cancer cells or treatment with a pharmacological inhibitor of CRAC channel resulted in reduced tumor metastasis in mice. The study further suggested that CRAC channel might act as a potential target for cancer therapeutics.

It has been shown that Orai3 and STIM1/STIM2 are involved in mediating SOCE in estrogen receptor positive breast cancer cells, while estrogen receptor negative breast cancer cells predominantly relied on the canonical Orai1/STIM1 CRAC channel to bring about SOCE [47]. The same group later demonstrated that silencing Orai3 could cause reduction in the anchorage-independent growth and the invasion of MCF-7 breast cancer cells (estrogen receptor positive), while having no such effect on MDA-MB-231 cells (estrogen receptor negative) [48]. Evidence for the role of Orai3 mediated SOCE in breast cancer cell growth and apoptosis resistance was provided by another study [49]. When compared with normal breast tissues and normal breast epithelial cell line (MCF-10A), the expression of Orai3 was found to be elevated in breast cancer tissue samples and breast cancer cell line (MCF-7). Furthermore, Orai3 silencing led to inhibition of MCF-7 cell proliferation and cell cycle arrest at G1 phase. Mechanistically, this was attributed to a decline in the expression of cyclins E/D1 as well as cyclin-dependent kinases (CDKs 4/2) and a concomitant accumulation of cyclin-dependent kinase inhibitor p21(Waf1/Cip1) and tumor suppressor protein p53. Silencing of Orai3, however, did not affect cell proliferation, cell cycle progression and the expression of cyclins D1/E, CDKs 4/2 and p21(Waf1/Cip1) in MCF-10A cells. Taken together, these reports underscore the significance of Orai3 CRAC channel as a selective therapeutic target for estrogen receptor positive breast cancers.

3.2 Gynecologic cancers

In a seminal study, Chen et al. [50] have identified the role of STIM1 in cervical cancer. Their clinical investigation revealed that STIM1 protein was overexpressed in the primary cervical cancer tissue of more than 70% of the patients in comparison to the adjoining non-malignant tissue from the same patient. Moreover, the expression of STIM1 correlated with tumor size and the clinical outcome. The five-year survival rate was considerably lower in cervical cancer patients with high expression of STIM1. This comprehensive study further demonstrated that silencing of STIM1 could inhibit proliferation of cervical cancer cells by inducing cell cycle arrest at the S and G2/M phases. In addition, the overexpression of STIM1 was found to promote migration of cervical cancer cells, while its knockdown reduced such invasive migration. Also, high STIM1 expression correlated with tumor growth, local invasion and angiogenesis in animal models.

It has also been shown that the activity of CRAC channel mediated SOCE fluctuates during cell cycle progression, where SOCE plays an important role in controlling G1/S cell cycle transition in cervical cancer cells [51]. Enhanced SOCE resulting from an increased expression of CRAC channel proteins ORAI1 and STIM1 has been reported in cisplatin-resistant ovary carcinoma cells, suggesting that CRAC channel could be a contributing factor toward therapy resistance in ovarian carcinoma [52].

3.3 Renal cancer

A study on clear cell renal cell carcinoma (ccRCC) has implied that SOCE mediated by CRAC channel may facilitate the development of ccRCC by enhancing cell proliferation and migration. It was observed that ccRCC cell migration and proliferation declined considerably upon knocking down Orai1 or STIM1 [53].

3.4 Colorectal cancer

Wang et al. [54] have observed the overexpression of STIM1 in patients of colorectal cancer (CRC). Interestingly, STIM1 overexpression in CRC was found to be related significantly with size of the tumor, lymph node metastasis, depth of invasion and the levels of carcinoembryonic antigen in serum ($p < 0.02$). The authors further reported that CRC cell motility gets enhanced by ectopic expression of STIM1, whereas STIM1 silencing via shRNA could prevent migration of CRC cells. In addition, their results indicated that STIM1 mediated CRC cell migration involved enhanced COX-2 (cyclooxygenase-2) expression and production of PGE2 (prostaglandin E2).

Another study which demonstrated that STIM1 induced metastasis in CRC cells, has also established poor prognosis in CRC patients to be associated with STIM1. Overexpression of STMI1 was observed in highly invasive CRC cell lines as well as in CRC tumor tissues compared to surrounding non-malignant tissues obtained from the same patient. Furthermore, STIM1 overexpression in CRC cells was found to promote metastasis and EMT (epithelial to mesenchymal transition), while STIM1 silencing with siRNA reduced both metastasis and EMT [55]. A separate study has implicated that the downregulation of STIM2 may contribute to apoptosis resistance in HT29 CRC cells [56].

3.5 Gastric cancer

In gastric cancer tissues, the expression levels of Orai1 and STIM1 have been reported to be higher than adjacent non-cancerous tissues. Such an increased expression of Orai1 and STIM1 was found to be associated with poor prognosis and high mortality rates in an investigation of 327 patients of gastric cancer. Moreover, this study showed that knockdown of Orai1 and STIM1 reduced the proliferation, migration and invasion of gastric cancer cells in vitro, as well as resulted in significant inhibition of tumor growth and metastasis in vivo. In addition, downregulation of Orai1 and STIM1 altered cell cycle and epithelial-mesenchymal transition (EMT) markers [57].

3.6 Liver cancer

Evidence for the higher expression of STIM1 in hepatocellular carcinoma cell lines than a normal hepatocyte cell line was provided by Yang et al. [58]. The study also reported elevated STIM1 expression in hepatoma tissues as compared to pre-cancerous tissues of the same patients. Another interesting observation was a five-fold elevation in STIM expression in HCC-LM3 cell line, known to possess a higher potential for migration, in comparison to other hepatocellular carcinoma cell lines.

CRAC channel activated SOCE has been implicated in chemoresistance of human hepatocellular carcinoma to 5-fluorouracil therapy. 5-fluorouracil has been shown to induce

autophagic cell death in HepG2 hepatoma cells by reducing the expression of Orai1 and consequently preventing SOCE. Moreover, increased expression of Orai1 was also observed in hepatocellular carcinoma tissues. Therefore, Orai1 expression may be considered as a prognostic marker of 5-fluorouracil sensitivity for hepatocellular carcinoma therapy and blocking CRAC channel mediated SOCE may sensitize hepatocellular carcinoma cells to 5-fluorouracil chemotherapy [59].

3.7 Pancreatic cancer

CRAC channel proteins ORAI1 and STIM1 have been demonstrated to play anti-apoptotic role in pancreatic ductal adenocarcinoma cells. It was observed that apoptosis induced by gemcitabine or 5-fluorouracil increased upon RNAi mediated silencing of ORAI1 and STIM1. The authors also reported relatively enhanced expression of Orai1 and STIM1 in several pancreatic ductal adenocarcinoma cell lines as compared to normal pancreatic ductal epithelial cells, suggesting that cancer cells possibly upregulate Orai1 and STIM1 as a mechanism to safeguard themselves from undergoing apoptosis [60].

3.8 Esophageal cancer

Tumor Orai1 expression was found to be considerably increased in comparison to adjacent non-cancerous esophageal tissues obtained from patients of esophageal squamous cell carcinoma (ESCC). This elevated expression of Orai1 correlated with poor overall survival and recurrence-free survival in ESCC patients. Further, downregulating Orai1 activity/expression was accompanied by reduced ESCC cell proliferation and migration in vitro and suppressed growth of tumor xenograft in vivo [61].

3.9 Lung cancer

Cisplatin has been shown to induce apoptosis through modulating STIM1 in non-small cell lung cancer (NSCLC) cell lines A549 and H460. The same study reported significantly higher expression of STIM1 in lung carcinoma tissue than in the adjoining non-cancerous lung tissue. Based on their findings, the authors concluded that STIM1 might play an important role in the development of lung cancer [62].

Ouadid-Ahidouch and co-workers [63] have also investigated the role of CRAC channel in NSCLC. They observed higher Orai3 expression in cancer tissues in comparison to non-cancerous tissues. Further, blocking CRAC channel pharmacologically or silencing Orai3 in NSCLC cell lines, namely NCI-H23 and NCI-H460, resulted in significant suppression of cell proliferation and arrest of cell cycle progression in G0/G1 phase. These phenomena were found to be associated with a reduced expression of cyclins D1/E and CDKs 4/2. In addition, knockdown of Orai3 reduced the phosphorylation levels of Akt, indicating that Orai3 CRAC channel mediated SOCE in NSCLC regulated tumorigenesis through Akt signaling pathway.

3.10 Glioblastoma

Ca^{2+} entry through CRAC channel has been shown to regulate the proliferation and apoptosis of glioblastoma cells. Use of CRAC channel blockers or siRNA knockdown of ORAI1 and STIM1 proteins, resulted in significant reduction in proliferation and a marked

increase in apoptosis of C6 rat glioma cells. Interestingly, a more significant antiproliferative effect was noticed in cells with siRNA knockdown of ORAI1 than those with STIM1 knockdown [64]. In another study, STIM1 silencing by siRNA suppressed the proliferation of U251 glioblastoma cells by triggering cell cycle arrest in G0/G1 phase via regulation of p21Waf1/Cip1, cyclin D1 and CDK4 (cyclin-dependent kinase 4). The antiproliferative effect of STIM1 downregulation was also seen in U251 glioblastoma xenograft tumor model [65].

Motiani et al. [66] reported that glioblastoma cells had elevated Orai1 expression associated with an upregulated SOCE when compared to human primary astrocytes. The authors further examined the functional significance of SOCE in glioblastoma by evaluating the effects of silencing STIM1 and Orai1 on cell proliferation and invasion. It was demonstrated that silencing of STIM1 and Orai1 led to a significant reduction in cell invasion in glioblastoma cells, but not in human primary astrocytes. The effects of STIM1 and Orai1 downregulation on glioblastoma cell proliferation were, however, found to be marginal. Furthermore, Zhu et al. [67] have also found elevated expression of Orai1 in multiple glioma cell lines and glioma tissues as compared to non-malignant brain tissues. They observed reduced glioma cell migration and invasion upon downregulating Orai1 expression or pharmacologically inhibiting CRAC channel. The results further indicated that phosphorylation of Pyk2 (proline-rich tyrosine kinase 2) could be essential in the pathway through which CRAC channel regulated migration and invasion of glioma cells.

3.11 Hematologic cancers

There is evidence to suggest that Orai1 and Orai2 are essential for the proliferation of HL60 leukemia cells and can regulate HL60 cell migration and FAK phosphorylation. Also, Orai2 was found to be overexpressed in HL60 cells [68]. Similarly, overexpression of Orai1 and STIM1 has been reported in multiple myeloma tissue and cell lines. The study also demonstrated that silencing of Orai1 or STIM1 or inhibition of SOCE resulted in a reduction of cell viability and caused apoptosis and cell cycle arrest in multiple myeloma cell lines [69].

3.12 Melanoma

Inhibition of CRAC channel activated SOCE in melanoma cells caused reduction in cell proliferation and migration. Results from the study suggested that CaMKII/Raf-1/ERK signaling pathway controlled the SOCE mediated proliferation and migration in melanoma cells. Moreover, STIM1 and Orai1 were reported to be highly expressed in human melanoma tissues and various melanoma cell lines [70]. Melanoma growth and invasion have also been reported to be controlled by STIM2 and Orai1 mediated Ca^{2+} entry [71].

3.13 Prostate Cancer

The exact role of CRAC channel is not clearly understood in prostate cancer, perhaps due to its heterogeneous nature. Orai1 mediated SOCE was reported to induce apoptosis in human prostate cancer cells by Flourakis et al., suggesting a tumor suppressive action of CRAC channel. Further, the study correlated low Orai1 levels with apoptosis resistant phenotype in prostate cancer cells [72]. Kappel et al. [73] have reviewed the dysregulation of CRAC

channel components in prostate cancer. Interestingly. It has been reported that the expression levels of STIM1 and Orai1 depend on the clinical stage of prostate cancer. Orai1 and STIM1 expression levels have been found to be elevated in early stages of prostate cancer, but they get reduced during the later castration resistant stages of prostate cancer [74].

4. Pharmacologic inhibition of CRAC channel as a cancer treatment strategy

Over the past three decades, our understanding of the molecular components and mechanism of action of CRAC channels has grown significantly. Concomitantly, the critical roles CRAC channels purportedly play in various human pathologies, including cancer, have been explored extensively. Moreover, the fact that CRAC channel proteins have been widely reported to be overexpressed in several types of human tumor tissues in contrast to their normal counterparts, has led to an increased realization that pharmacologically inhibiting CRAC channel mediated Ca^{2+} entry may possibly have significant implications for the development of cancer therapeutics. In other words, CRAC channels have emerged as potential targets for cancer therapy and this has ignited great interest in developing small molecule inhibitors to block this channel and its associated signaling in tumor cells.

Inhibitors of CRAC channel may act by either directly blocking the Orai pore of the channel or by targeting STIM or by interfering with Orai-STIM interactions, thereby regulating the overall activity of the CRAC channel. Several small molecule inhibitors targeting CRAC channel dependent Ca^{2+} entry have been developed over the years [10]. Some of them have shown promise as potential candidates for cancer therapy in preclinical studies [75]. Besides, these pharmacological inhibitors of CRAC channel have also proved quite helpful in understanding the roles of CRAC channel in tumorigenesis. A comprehensive description of the various challenges involved in the discovery and development of CRAC channel inhibitors has been provided by Stauderman [76]. Here, we discuss some of the CRAC channel blockers that have shown potential vis a vis cancer therapy. Figure 2 illustrates the chemical structures of these agents.

4.1 SKF-96365

SKF-96365, an imidazole compound, was shown to block SOCE in Jurkat cells [77] and rat basophilic leukemia cells [78]. It also reportedly blocked SOCE and NFAT nuclear translocation induced by STIM1 overexpression [79]. SKF-96365 was found capable of reducing tumor metastasis in animal models of breast cancer [22]. It also prevented angiogenesis and decreased the growth of cervical cancer xenografts in mice by blocking SOCE [50]. Inhibition of CRAC channel activity by SKF-96365 was shown to suppress the proliferation and migration of esophageal cancer cells in vitro and retard tumor growth in xenografted mice [61]. Interestingly, SKF-96365 was reported to be non-selective for CRAC channels as it could block other calcium channels as well [80]. Nevertheless, it should be noted that silencing or overexpressing Orai1 and STIM1 in the above-mentioned studies gave similar results which corroborate the observation that the effects of SKF96365 in those models stem from its ability to block Orai1-STIM1 CRAC channel.

4.2 2-APB

2-APB (2-aminoethyldiphenyl borate) has been reported to influence CRAC channel activity in a paradoxical manner, such that it inhibits activity of CRAC channel at high doses while stimulating it at lower doses. It was suggested that 2-APB, at high concentrations, was able to inhibit CRAC channel mediated Ca^{2+} entry by inhibiting STIM1 redistribution, while stimulating CRAC channel activity by enhancing STIM–ORAI1 interactions at low concentrations [81]. The effects of 2-APB on CRAC channel vary depending on the Orai homologues [82]. While at higher doses 2-APB acts as an inhibitor on Orai1 and Orai2, it stimulates Orai3 and reduces its Ca^{2+} selectivity independent of STIM1 or store depletion [83,84]. Multiple studies have shown that 2-APB has the potential to inhibit the proliferation of cervical [50], hepatoma [85] and gastric cancer cells [86]. It was also found to reduce the migration of CRC cells [54] and cervical cancer cells [87]. Despite exhibiting effective antitumor activities in multiple studies, 2-APB is considered not suitable for cancer chemotherapy, due to its non-specific and multi-target behavior. In an effort to overcome this problem, more selective 2-APB analogues, namely DPB-162AE and DPB-163AE, were developed, which proved to be 100 times more effective than 2-APB in blocking CRAC channel mediated SOCE [88]. However, the effect of these compounds on in vitro and in vivo cancer models are yet to be explored.

4.3 ML-9

ML-9 is an inhibitor of Akt kinase as well as STIM1, which is believed to block SOCE by interfering with STIM1 translocation [89]. ML-9 was shown to potently induce a concentration and Ca^{2+} dependent autophagic cell death in prostate cancer cells. It was further observed that ML-9 significantly promoted antitumor effects of docetaxel, which underscored its potential to be used in combination with existing anticancer chemotherapy [90].

4.4 Synta66

Synta66, developed by Synta Pharmaceuticals, is another selective blocker of CRAC channel activated SOCE. It has recently been reported to decrease EGF stimulated migration and proliferation in a breast cancer basal cell line model (MDA-MB-468) [91]. Despite promising initial findings, further investigations on Synta66 and its structural analogues, especially using in vivo models, are still needed.

4.5 CAI

CAI (Carboxyamidotriazole), a synthetic inhibitor of CRAC channel mediated Ca^{2+} influx, has been shown to decrease cell proliferation, invasion, migration and matrix metalloproteinase production in several head and neck squamous cell carcinoma cell lines, which has helped in establishing CRAC channel activated calcium signaling as a novel target for head and neck cancers [92]. CAI also demonstrated antiproliferative effect on hepatoma cell lines almost ten-fold greater than 2-APB [78]. Anti-angiogenic effects of CAI have also been reported [93]. Further evidence for the anti-angiogenic properties were provided by a study where CAI was shown to inhibit the proliferation and tubulogenesis of endothelial progenitor cells from renal cellular carcinoma patients [94].

Besides preclinical studies, CAI has also been investigated in multiple clinical trials. In one such phase II trial, CAI was found to enhance disease stabilization in patients with relapsed epithelial ovarian carcinoma and was suggested to be useful as a maintenance therapy owing to its limited toxicity profile [95]. However, in another Phase II trial in patients with metastatic renal cell carcinoma, CAI was reported to have little to no effect on response rate and disease progression [96]. Promising activity and effective brain penetration of CAI orotate was observed in a recent multicenter Phase IB study, which led to the conclusion that CAI orotate could be safely used in combination with temozolomide or chemoradiation in hard to treat glioblastoma and anaplastic gliomas [97].

4.6 RP4010

RP4010 is a new and potent CRAC channel inhibitor developed by Rhizen Pharmaceuticals SA. Cui et al. [98] have examined the anti-cancer effects of RP4010 in esophagus squamous cell carcinoma. The authors demonstrated that RP4010 caused reduction in intracellular Ca^{2+} oscillations, concomitant with cell growth inhibition and cell cycle arrest at G0/G1 phase in cultured esophageal cancer cells as well as a decrease in tumor growth in vivo. Recently our lab has also published a study on the effect of CRAC channel inhibition by RP4010 in pancreatic ductal adenocarcinoma models. RP4010 proved to be effective in suppressing the proliferation of various pancreatic cancer cell lines and was also able to reduce the growth of patient derived tumor xenograft in mice, more potently when combined with gemcitabine and nab-paclitaxel [99]. These findings, therefore, imply that CRAC channel inhibition can have translational relevance as a potential chemotherapy or adjuvant therapy in cancers such as esophagus squamous cell carcinoma and pancreatic ductal adenocarcinoma. However, the exact mode of binding and the mechanism underlying inhibitory action on CRAC channel components needs to be further elucidated. RP4010 continued to be under clinical development until very recently. A multicenter Phase I/Ib clinical trial for evaluation of the safety and efficacy of escalating doses of RP4010 in patients with relapsed or refractory non-Hodgkin's lymphoma had to be stopped after a review of the pharmacokinetic data (ClinicalTrials.gov Identifier: NCT03119467).

5. Conclusion

CRAC channel regulates intracellular Ca^{2+} homeostasis in non-excitable cells through mediating SOCE which is triggered in response to depletion of Ca^{2+} from the endoplasmic reticulum stores. STIM proteins sense the loss of Ca^{2+} from endoplasmic reticulum and their subsequent coupling with plasma membrane bound Orai proteins forms a functional CRAC channel that allows the influx of Ca^{2+} into the cell. The critical role of CRAC channel mediated Ca^{2+} entry in promoting cell proliferation, cell cycle arrest, resistance to apoptosis, invasion and metastasis in several types of cancers has been demonstrated in numerous independent studies. Moreover, there is ample evidence that the expression of CRAC channel proteins, Orai and STIM, is upregulated in various types of cancer cells and patient tumor tissues as compared to normal cells and non-cancerous tissue samples, respectively.

The established role of CRAC channel mediated SOCE in cancer progression also has some strong therapeutic implications, as it gives rise to the possibility of pharmacologically

inhibiting CRAC channel to selectively target cancer cells. This anticancer therapeutic potential of targeting CRAC channel gets strengthened by the observation that pharmacological inhibition of CRAC channel or silencing of CRAC channel proteins can effectively suppress tumor growth and metastasis in several cancer models. In addition, CRAC channel inhibition has been shown to enhance the effect of standard of care chemotherapy drugs. A number of CRAC channel blockers have shown anticancer effects in preclinical studies, but a majority of them did not manage to reach the clinical trial stage, mainly because of their toxicity and off-target effects. Nonetheless, there are some selective CRAC channel blockers that hold promise for future drug development.

6. Expert Opinion

The process of SOCE was first described by J. W. Putney in 1986 [100] as the depletion of Ca^{2+} stores directly resulting in activation of Ca^{2+} channels in the cell membrane, but the mechanism of SOCE remained largely enigmatic for over two decades until STIM and Orai proteins were identified as the key components for CRAC channel activation. Therefore, our understanding of CRAC channels is relatively recent. Regardless of this, CRAC channel mediated SOCE and its function in various cellular processes has been a subject of considerable interest. Moreover, the implications of CRAC channel activity in various human diseases, including cancer, has garnered a lot of attention. As the field evolved over the last two decades and new findings continued to emerge, our knowledge about this Ca^{2+} channel and its associated signaling pathways has kept on growing. Despite dissecting much of the molecular basis of CRAC channel activation and the functional association these channels possibly have with tumor development, there are still gaps in our understanding of the molecular mechanisms through which CRAC channels modulate tumor growth and metastasis. Nonetheless, the pleiotropic roles CRAC channel plays in tumorigenesis makes it an attractive target for cancer therapy. Yet, there is a long way to go before the full potential of blocking CRAC channel activity is harnessed as an anticancer therapeutic strategy.

So far, no inhibitor of CRAC channel mediated SOCE has been approved by FDA for clinical use in cancer treatment. But, a few CRAC channel inhibitors have succeeded in entering clinical trials. An increased understanding of CRAC channel activation and mechanisms, together with the development of new techniques to manipulate channel activity and a growing interest shown by several pharmaceutical companies, will hopefully accelerate the development of novel, more potent drug candidates that may specifically target CRAC channels for cancer therapy. More importantly, developing drugs that can specifically target a particular CRAC channel component may prove to be more effective in augmenting the selectivity and minimizing off-target effects. In this regard, structure based rational designing of drugs targeting CRAC channel proteins with greater efficacy and specificity may be expected to come up with promising leads for cancer therapy in the future. It is also worth mentioning that most of the studies on CRAC channels have focused on STIM1-Orai1 complex, which is undoubtedly the best characterized CRAC channel. However, the role of other STIM and Orai isoforms in cancer development and drug resistance remains understudied. Consequently, no CRAC channel inhibitor specific for these underappreciated CRAC components has been developed.

Since CRAC channel proteins are expressed on normal cells as well, another challenge in drug development would be to minimize toxicity to normal cells when CRAC channel blockers are administered systemically in cancer patients. This may be achieved by a CRAC channel inhibiting drug candidate that specifically targets cancer cells by recognizing their overexpressed STIM-Orai signature. Moreover, overexpression of STIM and Orai proteins observed in several types of cancers not only aid in selective targeting of cancer cells by CRAC channel blockers but can also have implications for the adverse prognosis of the cancer. For instance, overexpression of Orai3 in the tissues of lung adenocarcinoma patients [63] can be a prognostic marker for the disease and any prospective CRAC channel blocker which may be specific for Orai3 can be used to selectively target lung cancer cells. Therefore, identifying the specific type of CRAC channel components that contribute to tumor development in an individual cancer patient or a certain patient population can pave the way for possible selective targeting of cancer in a precision medicine approach.

It may be noted that so far only the overexpression of CRAC channel proteins has been widely considered to play the major role in cancer development. However, there is emerging evidence that certain mutations in Orai can result in its constitutive activation and consequent Ca^{2+} influx. These mutations have been found in large-scale cancer genomics datasets for dysfunctional Orai1 mutants [101]. More such studies in the future may lead to a paradigm shift in our understanding of the functional roles that proteins of the CRAC channel complex play in cancer development and progression. This may also provide new vistas for designing drugs aimed at targeting specific chinks in the armor of CRAC channel machinery.

CRAC channel proteins are also reportedly overexpressed in response to treatment with certain standard of care drugs, thereby contributing to drug resistance in various cancers. The use of CRAC channel inhibitors as adjuvant to chemotherapy can overcome this drug resistance and can even possibly enhance the efficacy of the chemotherapeutic drug. In fact, studies from our lab have recently demonstrated that CRAC channel inhibitor RP4010 was able to synergize with gemcitabine and nab-paclitaxel in pancreatic ductal adenocarcinoma preclinical models [99]. Even though the Phase I/Ib clinical trial of RP4010 as a monotherapy in non-Hodgkin’s lymphoma proved unyielding, the way forward would be to use RP4010 or any comparable CRAC channel blocker in combination with existing chemotherapy or even immunotherapy in the future clinical trials.

Further, recombinant Orai1 monoclonal antibodies have been shown to bind Orai1 strongly and specifically, leading to the internalization of Orai1 proteins, thereby causing loss of SOCE activity [102]. Such potential anti-Orai based therapy has been used for immune disorders. A similar application for cancer therapy holds promise and needs to be explored in future preclinical and clinical studies. Only time will tell if the safety and efficacy of CRAC channel blockers get established by the yet to be initiated clinical trials. Meanwhile, preclinical studies continue to shed more light on the myriad roles that CRAC channels play in carcinogenesis and suggest the potential therapeutic value of targeting these channels in cancer.

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Declaration of interest

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* = of interest, ** = of considerable interest

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Article Highlights:

- CRAC channel is responsible for maintaining cellular Ca^{2+} homeostasis.
- CRAC channel is activated by the coupling of channel pore forming Orai protein and endoplasmic reticulum Ca^{2+} sensing STIM protein.
- CRAC channel plays vital roles in cancer cell proliferation, tumor growth, metastasis and tumor neovascularization.
- CRAC channel proteins are overexpressed in cancer cells and tissues.
- Pharmacological inhibition of CRAC channel represents a promising strategy for anticancer therapy.

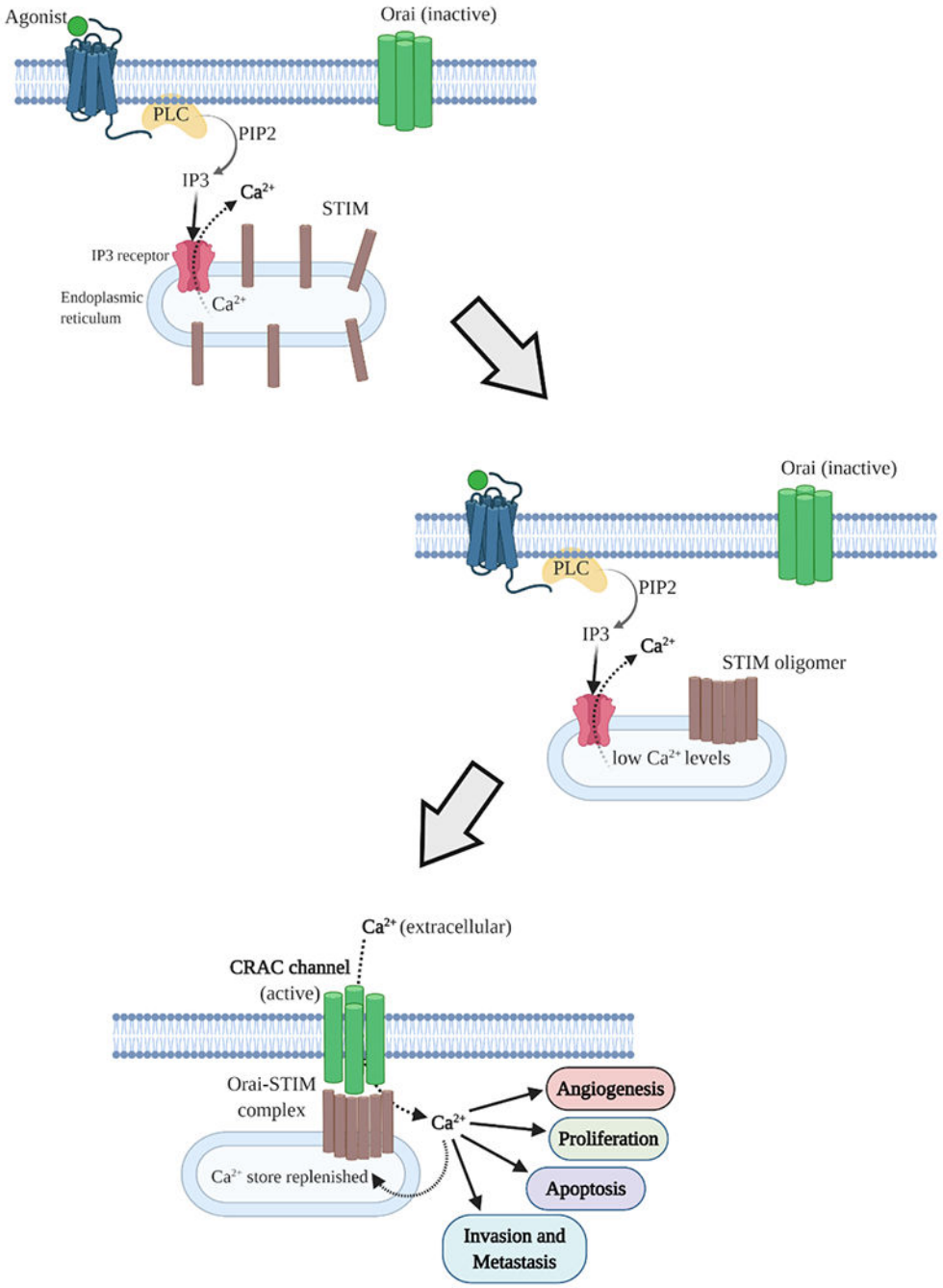

Figure 1.

Schematic diagram showing the mechanism of CRAC channel activation. Binding of their corresponding agonists (ligand) stimulates diverse plasma membrane receptors which activate Phospholipase C (PLC). PLC then generates inositol triphosphate (IP3) from phosphatidylinositol-4,5-bisphosphate (PIP2). IP3 binds to the IP3 receptor on the endoplasmic reticulum and triggers Ca^{2+} efflux. STIM proteins, which are evenly distributed on endoplasmic reticulum membrane at resting state, sense the drop in Ca^{2+} levels in endoplasmic reticulum lumen and undergo rapid conformational changes that enable them to

bind with Orai proteins. This results in the functional activation and opening of CRAC channel through which extracellular Ca^{2+} enters the cell. Sustained increase in intracellular Ca^{2+} not just refills the endoplasmic reticulum Ca^{2+} stores but also activates downstream signal transduction pathways that regulate various cellular processes.

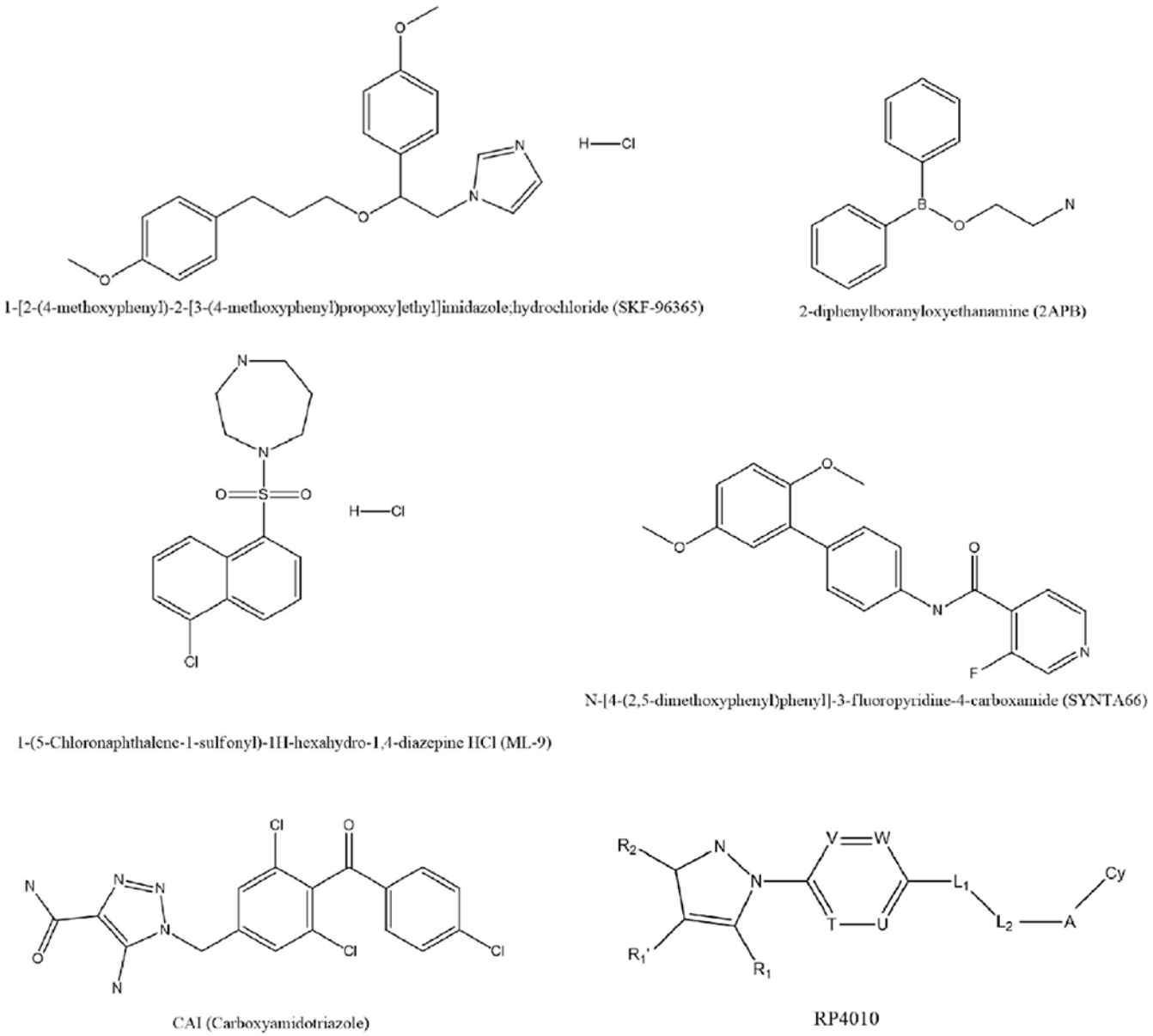

Figure 2.

Chemical structures of CRAC channel inhibitors that have shown potential anticancer properties.

Table 1:

Altered expression of CRAC channel proteins and their reported effects in different types of cancers

Cancer type	Cancer model	Change in expression of CARC channel protein	Effect	Reference
Breast	Microarray profiles of 295 human breast tumors	High STIM1, low STIM2	Associated with poor prognosis	45
Breast	MDA-MB-231, MC-7, T47D, BT-483	Elevated Orai1	Remodeling of Ca^{2+} influx associated with invasive stimuli	45
Breast	MCF-7 cells and cancerous breast tissue samples	High Orai3 expression	Promotes cell proliferation, cell cycle progression and apoptosis resistance	49
Cervical cancer	primary cervical cancer tissues	Overexpression of STIM1	Reduces five-year survival rate; Promotes tumor cell growth, cell cycle progression, migration, invasion and angiogenesis	50
Ovarian cancer	A2780cis (cisplatin resistant) cells	High Orai1 and STIM1 expression	Contributes to therapy resistance	52
Renal Cancer	ccRCC tissues	High expression of Orai1	Promotes cell proliferation and migration	53
Colorectal cancer	Patient tumor tissue specimen	STIM1 overexpression	Increases tumor size, invasion and migration	54
Colorectal cancer	SW620 and LOVO cells	STIM1 overexpression	Promotes metastasis and EMT	55
Colorectal cancer	Tissue microarray of 90 CRC patients	STIM1 overexpression	Indicative of poor prognosis	55
Gastric cancer	Tumor tissues from 327 GC patients	High Orai and STIM1 expression	Correlates with frequent recurrence and high mortality rate	57
Liver cancer	Hepatoma tissues and HCC cell lines	Higher expression of STIM1	Correlates with high metastatic potential in patients and higher migration ability in cell lines	58
Liver cancer	Hepatocarcinoma tissues	Elevated Orai1 expression	Serves as predictor of 5-FU sensitivity	59
Pancreatic cancer	Panc-1 cells	High expression of Orai1 and STIM1	Contributes to apoptosis resistance	60
Esophageal cancer	Tumor tissues from ESCC patients	Elevated expression of Orai1	Linked to poor overall and recurrence-free survival	61
Lung cancer	NSCLC tumor tissues	Higher STIM1 expression	Promotes tumorigenesis	62
Lung cancer	Tumor samples from 60 lung adenocarcinoma patients	Orai3 overexpression	Correlates with high tumor grade	63
Glioblastoma	U251 cells	Higher expression of STIM1	Promotes cell proliferation and invasion	65
Glioblastoma	Primary human cell lines established from glioblastoma surgical samples	Increased Orai1 levels	Promotes cell invasion	66
Glioblastoma	Tumor tissues from 61 glioma patients	Elevated Orai expression	Correlates with high tumor grade	67
Glioblastoma	Human glioma cell lines U251, SNB19, U87, and LN229	Elevated Orai expression	Promotes cell migration and invasion	67
Leukemia	HL60 cells	High expression of Orai2	Promotes cell migration	68
Multiple myeloma	Tumor tissues from 60 Multiple Myeloma patients	Increased expression of STIM1 and Orai1	Associated with poor prognosis	69
Multiple myeloma	KM3 and U266 cells	Increased expression of STIM1 and Orai1	Promotes cell growth and apoptosis resistance	69