# Therapeutic Prospective of Targeting Calcium Homeostasis in Cancer – A Review

By

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy

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## Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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# Approval

The thesis/project titled “Therapeutic prospective of targeting calcium homeostasis in cancer - a review” submitted by Tasnova Kamal (15146017) of summer 2019 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on August 26, 2019.

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## Ethics Statement

## Abstract

Cancer is recognized as the major concern in today's world. Cancer is a condition or a group of diseases in which cells are abnormally divided. In the past century, the understanding and treatment of cancer has improved a lot and treatment such as chemotherapy, radiation therapy, surgery etc. have emerged. However, these are not enough to meet the desirable expectations and there is still need of new approaches for better treatment. A series of current reports described that an imbalance in intracellular $Ca^{2+}$ homeostasis may cause cancer in the human body. So, to treat cancer, targeting derailed $Ca^{2+}$ signaling is being explored more recently. This review has discussed about some important $Ca^{2+}$ channels, transporters and $Ca^{2+}$ ATP-ases that have been found to be altered in cancer patients. It also involves the research and effort of the scientists towards the evaluation of the blockers, inhibitors or regulators for $Ca^{2+}$ channels/transporters or $Ca^{2+}$-ATPase pumps as potential anti-cancer drugs.



**Keywords:** cancer therapy; calcium homeostasis; $Ca^{2+}$ signaling; apoptosis; channels; drugs.

## Dedication

Dedicated to my parents and respected teachers.

## Acknowledgement

I would like to begin by thanking the Almighty Allah, our creator, the source of our life, strength, knowledge, wisdom, blessings and mercy. All praises to the Almighty Allah for blessing me with immense patience and strength to complete this project. This project would not have been completed without the support of the people who are recognized here.

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# Table of Contents

# List of Tables

# List of Figures

## List of Acronyms

| | |
|---|---|
| SV | Simian Virus |
| VOCC | Vessel Operating Common Carrier |
| TRP | Transient Receptor Potential |
| SOCE | Store Operated Calcium Entry |
| STIM | Stromal Interaction Molecule |
| CRC | Colorectal Cancer |
| mRNA | Messenger Ribonucleic acid |
| PMCA | Plasma Membrane Calcium ATP-ase |
| SOCS | Suppressor of cytokine signaling |
| MCF-7 | Michigan Cancer Foundation-7 |
| IP3 | Inositol 1,4,5-Trisphosphate |
| NFAT | Nuclear Factor for Activated T-cells |
| $[Ca^{2+}]_{CYT}$ | Cytoplasmic-free Calcium |
| Pca | Prostate cancer |
| SERCA | Sarco/endoplasmic reticulum $Ca^{2+}$-ATPase |
| ERK | Extracellular-Signal-Regulated Kinase |
| SCLC | Small Cell Lung Carcinoma |
| VEGF | Vascular Endothelial Growth Factor |
| CAI | Carboxyamidotriazole |
| AMP | Adenosine monophosphate |
| TG | Thapsigargin |
| PSMA | Prostate Specific Membrane Antigen |
| CRAC | Calcium release-activated channels |
| CDK4 | Cyclin-dependent kinase 4 |

# Chapter 1

# Introduction

## 1.1 What is cancer?

Cancer is a group of diseases in which body cells divide abnormally and uncontrollably, with the potential to spread to nearby tissues. Cancer cells can also expand through the blood and lymph systems to different parts of the body. Cancer has been known since ancient times but from the middle of the 20$^{th}$ century some of the most significant advances in scientists' understanding of it have been made. Cancer is caused because of the irregular mechanisms of cell growth and cell death. Tumor develops from these irregularities in the amount of cell growth associated with obstruction of cell apoptosis, that gradually leads to variation in tissue homeostasis and the cell growth process becomes out of control. Proliferation and apoptosis involve different pathways and molecular actors. Proliferation relies on cyclin dependent protein kinase regulators of the cell division cycle while apoptosis primarily depends on caspases cysteine proteases executing a cell death programme. At an early preneoplastic progression stage, cells have a sharp increase in apoptosis activation in low serum conditions when it is compared to normal cells. Moreover, at a later progression stage, cells become tumorigenic and show a dramatic decrease in apoptotic cell death. Although these data provide insight into when changes in the regulation of apoptosis homeostatic processes might occur during tumor progression, it is necessary to extend these studies to determine how a cell might modulate signals to block apoptosis. (Dubois, Abeele, & Prevarskaya, 2013)

## 1.2 The need of new approaches in cancer treatment

After the middle of 20th century scientists came up with some significant enhancement in cancer therapy, mainly through the establishment of methods for prompt and precise diagnosis, selective surgery, radiation therapy, chemotherapeutic drugs and targeted therapies. The treatments are dependent on the type of cancer the patient has and how advanced it is. Rather than treating the cancer with one therapy, most of the people receive combination of treatments, such as surgery with chemotherapy and/or radiation therapy. Surgery is a procedure where the cancer is removed from the body and radiation therapy is a process where high radiation doses are used to shrink tumor or to kill the cancer cells. In addition to that, another most common cancer therapy is using chemotherapeutic drugs. These drugs are used to kill cancer cells from the body. On the other hand, there are also some other therapies such as immunotherapy, targeted therapy, hormone therapy, stem cell transplant etc. Though in the last decade there has been a decrease in the mortality rate for all types of cancers due to early diagnosis, preventive actions, improved lifestyle, very few cancers can actually be cured by pharmacological treatment. Though the advancement in the understanding of cell proliferation and dissemination are satisfactory, this expectancy is not being met by the drugs currently available in the market (Agency & Evaluation, 2003). Moreover, the drugs that are licensed up in the end of the year 2000 are not innovative enough (Agency & Evaluation, 2003). So it can be said that, despite a massive improvement in cancer treatments, there is still need for better approaches for the early diagnosis and better treatment of this life threatening disease.

## 1.3 Reasons behind targeting calcium homeostasis in cancer

The laboratory investigations are aimed at understanding the mechanisms and reasons for the development of cancer and the researchers are optimistic that the disease can be controlled. They

have gained a fundamental understanding of cancerous cells by major breakthroughs in cell biology, genetics and biotechnology. Through the collection of different results from studies, it was shown that alteration of intracellular $Ca^{2+}$ homeostasis is seen in cancer cells, and this could be involved in tumor initiation, angiogenesis, progression and metastasis. Therefore, for cancer therapy, targeting derailed $Ca^{2+}$ signaling has become an emerging research area(Costa, 2019).

There is some evidence that $Ca^{2+}$ signaling is involved in apoptosis, although the role of $Ca^{2+}$ in this process is still unclear. Calcium homeostasis is defined as the regulation of calcium ion concentration in the extracellular fluid $[Ca^{++}]_{ECF}$. The calcium ions have a stabilizing effect on voltage-gated ion channels, so this parameter is tightly controlled. As an example, when $[Ca^{++}]_{ECF}$ becomes too low (hypocalcemia), voltage-gated ion channels start to open spontaneously and this results in hyperactive nerve and muscle cells causing a spasm. This involuntary muscle spasm due to low $[Ca^{++}]_{ECF}$ is called hypocalcemic tetany. On the other hand, in too high $[Ca^{++}]_{ECF}$ (hypercalcemia), voltage-gated ion channels do not open as easily, and that causes depressed nervous system function. In hypercalcemia, calcium and phosphate ions combine together and form calcium phosphate deposits causing calcification and stones in blood vessels and in the kidneys (Hormones, 2017) (Stewart, Yapa, & Monteith, 2015).

It was originally proposed that the primary importance of $Ca^{2+}$during apoptosis is an increase in cytosolic free $Ca^{2+}$concentration, $[Ca^{2+}]$and that $Ca^{2+}$ was required for the activation of an endonuclease responsible for DNA nucleosomal cleavage. However, in the absence of any detectable rise in $[Ca^{2+}]$, apoptosis has been observed in some systems. In fact, growth factor withdrawal that induces apoptosis, can be suppressed by increasing $[Ca^{2+}]$. Furthermore, whether a cell will survive or die can be dictated by high nuclear calcium level maintenance.

Interestingly, studies suggesting that transformation is associated with modulations of $Ca^{2+}$ sensitive signaling pathways have shown that lowering of extracellular [$Ca^{2+}$] resulted in a reversible block in the cell cycle of normal cells, whereas SV4O-transformed cells continued to proliferate in low [$Ca^{2+}$] levels (Preston, Barrett, & Biermann, 1997) (Farfariello, Iamshanova, Germain, Fliniaux, & Prevarskaya, 2015)

There are some other reasons that is why calcium signaling can be targeted for cancer therapies. The amount of intracellular $Ca^{2+}$ ions in human body is plentiful and they work as a second messenger. These $Ca^{2+}$ ions take part in different basic cell activities such as expressing genes, controlling cell cycle, motility, autophagy and apoptosis. Normally cytosolic $Ca^{2+}$are kept to very low amount (~$10^{-7}$ mol/L). However, sometimes different cellular organs inside the cell membrane secrete a little amount of $Ca^{2+}$(~$10^{-5}$ mol/L). This particular $Ca^{2+}$ or $Ca^{2+}$ influx from extracellular sources stimulate downstream signaling pathways. Though this elevated $Ca^{2+}$ level is for specific areas, this alteration of ions can generate a wave and can affect outlying areas too as they have the capability to diffuse across the wall. If this situation is maintained for a prolonged time it can be toxic and results in cell death. This is why regulating $Ca^{2+}$ signals are very important and it must be balanced in different forms such as through spikes, waves or oscillation (Cui, Merritt, Fu, & Pan, 2017).

The oscillation of $Ca^{2+}$ inside the cell membrane has the ability to pass on biological data. For instance, former studies indicate that, this type of signal is responsible for the uncontrolled proliferation in esophageal cancer. These oscillations have precise frequency, amplitude, duration and these form a specific $Ca^{2+}$ code. These codes are responsible for actuating various transcription factors such as gene transcription, cell growth, migration etc. In addition to this, the downstream effectors inside the cell membrane take part in decrypting those oscillatory forms.

These effectors such as calmodulin (CaM), nuclear factor of activated T-cells (NFAT), nuclear factor-$\kappa$B (NF-$\kappa$B), calmodulin-dependent protein kinase II (CaMKII) and calpain etc. have their own differences in 'on and off rates' for $Ca^{2+}$ and have the capacity to gradually actuate various cell activities. Moreover, different marked sites are usually taken up by some kinases and enzymes which are maintained by $Ca^{2+}$. This oscillation inside the cell membrane is responsible for lowering $Ca^{2+}$ threshold for signal transduction (Cui et al., 2017).

When normal $Ca^{2+}$ signaling is imbalanced due to interference it can lead to formation of harmful phenotypes. In tumors, different cell activities are seen to be malfunctioned such as, the cells grow uncontrollably, they evade apoptosis, somehow dodge the immune system etc. The main culprit behind this is the alteration of $Ca^{2+}$ signaling system on their own by the tumor cells. These abnormal phenomena are also correlated with the overexpression or irregular actuation of $Ca^{2+}$ channels, transporters, $Ca^{2+}$ATP-ase. So, targeting these particular things can be useful in cancer therapies. Therefore, blocking $Ca^{2+}$ channels, inhibiting ATP-ase pumps etc. may bring desired results in cancer treatment (Cui et al., 2017).

## 1.4 Rationale of the study

Cancer is such a disease which does not have an exact treatment right now. The previous treatment procedures are not enough to treat this deadly disease as they have their limitations. Moreover, the mortality rate of cancer is becoming high day by day. Therefore, for better life and better treatment new approaches are needed and targeting calcium homeostasis can be a very good option. This study is to understand how the calcium homeostasis is imbalanced in different

types of cancers and how calcium channels, transporters and pumps are related to this and their potentiality to be used as anti-cancer drugs.

## 1.5 Aim of the study

The aim of this review is to aid the search for discovering new methods, targets and drugs to treat cancer by targeting $Ca^{2+}$ channels or transporters. As cancers can be seen as an area of high unmet need, targeting $Ca^{2+}$ channels are one of the new and emerging approaches to treat cancer and meet this demand. Thus, this review is very important.

## 1.6 Objectives of the study

The objective of this review is to highlight the importance of calcium homeostasis, which has been reported to be changed in patients suffering from cancer. The other objectives are to support research and to nourish interest so that the scientists concentrate more to understand the mechanisms that are behind maintaining $Ca^{2+}$ signals in various forms of cancer.

# Chapter 2

# Results and discussion

## 2.1 Calcium and cancer

Tumors hamper the calcium homeostasis in the body and this imbalance gradually leads to irregular bone metabolism. Osteolytic metastases and osteoblastic metastases are included in bone abnormalities which lead to increased bone formation. When the calcium concentration becomes higher it is called hypercalcemia and this results in various symptoms. However, cancer is associated with hypocalcemia more frequently than hypercalcemia. The major mechanism of malignant hypercalcemia is increased bone resorption. Because of the evolution in this field, the scientists could gather more ideas about normal bone metabolism and calcium regulation. This information has made it easier to understand the mechanisms behind the imbalanced calcium homeostasis in cancer patients. Upcoming advancement can be discovered by elucidation of the cellular and molecular mechanisms of bone resorption in cancer (Dimitrov, Salehi-tabar, An, & White, 2013).

## 2.2 $Ca^{2+}$ remodeling in cancer

From various researches and studies, researchers have become increasingly confident that, changes in intracellular $Ca^{2+}$ homeostasis (remodeling) may contribute to critical cancer hallmarks. Different groups of cells have different specific activities like proliferation, invasion, migration etc. and these are controlled and monitored by intracellular $Ca^{2+}$. There are several evidences of altered vessel operating common carrier (VOCC) expressions in different types of cancers for instance prostate, gastric and colorectal cancers. In addition to that, the transient

potential receptor (TRP) channels are also seen to change their expressions in breast, ovary, kidney cancers and glioma. Moreover, store operated calcium entry (SOCE) and its underlying currents and molecular players are sometimes responsible for cancer. Significant proteins like STIM1 and Orai1 are associated with Icrac and SOCE and this involvement takes part in the metastasis and migration of breast cancer cells (Villalobos et al., 2017).

In case of cervical cancer this particular protein (STIM1) is responsible for cell growth and cell migration. In approximately 70 out of 100 cervical cancer cases, STIM1 is seen to be elevated and this leads to metastasis. Furthermore, STIM1 also has a significant part in glioblastoma and both STIM1 and Orai1 are associated with the disruption of glioblastoma. So silencing or inhibiting these can be a potential target in cancer therapy, as it may prevent glioblastoma cell proliferation in humans by stimulating G0/G1 phase arrest. Orai3 is sometimes involved in prostate and breast cancer. Therefore, these results provide evidence that atypical expression of molecular players in SOCE can lead to different types of cancer. (Villalobos et al., 2017)

Recently cancer is one of the major burdens of the world. In some of the countries it ranks number two in the list of ‘most common death reasons’. According to the National Cancer Institute (USA) it is increasing day by day. In 2012, among 14.1 million new cancer cases, the worldwide death rate was 8.2 million. Moreover, from the information of the World Health Organization (WHO) it is found that, new cancer cases will be increased to 24 million whereas the death rate will be 14.5 million a year by 2035.In addition to that, the World Health Organization also reminded that, by 2020 the rate of cancer patients will go higher globally by 50% (Mini-overview, 2017).

Previously most types of cancer were treated by using chemotherapy. However, the increasing occurrence of intrinsic or acquired drug resistance is a huge obstacle on the way of its success. So, often failure of the chemotherapy treatment occurs because of the drug resistance. A cross resistance to multiple drugs can occur when the patient is resistant to one particular anti-cancer drug or more than one drug. Any patient can be resistant to a specific drug even before the cancer appeared or it can also be a result of medications (Kartal-yandim, Adan-gokbulut, & Baran, 2015). Moreover, cancer cells sometimes adopt different mechanisms to resist chemotherapy. These cells are capable of modifying their metabolic activities, altering their own structure so that the drugs cannot target the tumor specific site and redefining the genes. As a result, the cancer cells become resistant to apoptosis or necrosis. This is why the whole world is concentrating on discovering new drugs and mechanisms that will treat cancer (Mini-overview, 2017).

For this purpose, $Ca^{2+}$ signaling can be targeted as a new form of therapy. $Ca^{2+}$ targeting drugs increase apoptotic cell death and treat cancer. To make this evident, scientists have done some experiments on these $Ca^{2+}$ channels. Among them thapsigargin (TG), which is a $Ca^{2+}$ inhibitor was tested thoroughly. The test was conducted on human hepatoma cells of BEL-7404 cell line and the results were evaluated both on flow cytometry and electronic microscope. With increasing dose, the extent of apoptosis also increases in human BEL-7404 cells, if TG (from 0.01 μM to 1 μM) is treated for 48 hours in serum free condition. On the other hand, the enhancement of the rate of apoptosis was not detectable even when 1μM TG was treated in fetal calf serum (13%). Moreover, prolonging the time of the condition without serum by 48 more

hours, it was found that, TG stimulated apoptosis rate has increased from 6.01% to 20.83%. Therefore, it is exhibited that, serum starvation does not affect programmed cell death in BEL-7404 cells. These results lead to the conclusion that, serum-starvation and TG treatment have additive effects on apoptotic cell death (Oncology, 1995).

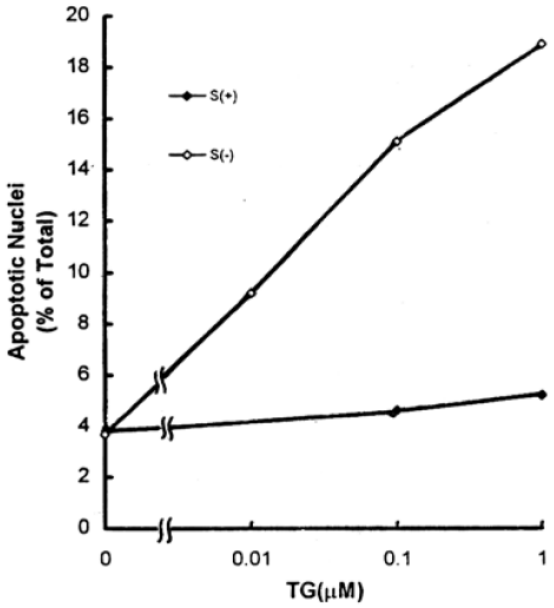


*Figure 1: Dose dependence of TG induced apoptosis in BEL-7404 cells.*

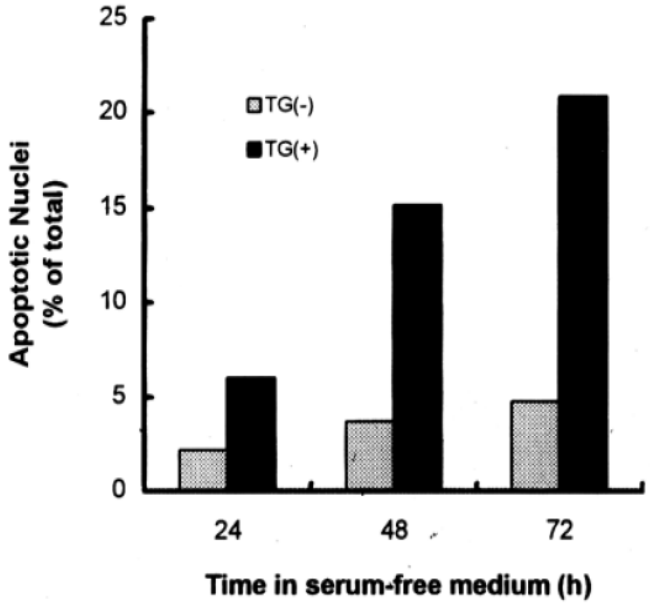


*Figure 2: Effect of serum starvation on TG induced apoptosis in BEL-7404 cells.*

Furthermore, in hepatoma cells, TG induced apoptosis was detected by electron microscope. It has shown that the increment rate of sub-G1 cells associated with lower DNA content is more than in G1 phase cells. Later, it is exhibited by flow cytometric analysis. In addition, more than 95% of cells excluded the charged dye, trypan blue, under similar conditions was demonstrated in an assay of plasma membrane integrity. This information makes it evident that, TG stimulates apoptosis in BEL-7404 cells(Oncology, 1995).

Moreover, scientists also studied the anti-tumor effects of a compound named amlodipine. It is a dihydropyridine $Ca^{2+}$ channel blocker. This particular compound is tested on human epidermoid carcinoma A431 cells both in vivo and in vitro. Whenever, a cell cycle goes on it needs $Ca^{2+}$ in different stages. So, the effects of amlodipine in A431 cells' distribution is studied. From the

analysis in flow cytometry, it is revealed that treating cells with amlodipine (20– 30 mM, for 24 hour) causes arrest of the cell cycle at G1 stage. G1 arrest involves many alterations such as phosphorylation of some protein called retinoblastoma (pRB) is reduced, cyclin D1 and kinase 4 which is regulated by cyclin are also reduced and $p21^{Waf1/Cip1}$ is overexpressed. It also involves a regulator that helps the conversion from G1 to S stage, a protein that can prevent CDK/cyclin complex. From the in vitro assay, it is found that amlodipine has the ability to lower CDK-2, CDK-4 and also the functions of kinase that is regulated by cyclin E and cyclin D1. Additionally, there are other dihydropyridine derivatives such as nicardipine, nimodipine that have the ability to reduce overexpression of cyclin D1 protein and functions regulated by CDK kinase. However, despite being in the same class, nifedipine, does not show any antiproliferative characteristics on A431 cells. These information shows that, these $Ca^{2+}$ channel blockers prevent cancerous cell growth and DNA synthesis in different cancer cells. This study must be further validated with more in vitro and in vivo study (Yoshida, Ishibashi, & Nishio, 2007).

## 2.3 $Ca^{2+}$ remodeling in conferring apoptosis

In many original studies the $Ca^{2+}$ dependence of apoptosis is defined properly (Orrenius, Zhivotovsky, & Nicotera, 2003). Because of enormous entry and extensive release, cytosol is overloaded by $Ca^{2+}$ and can later on headway through mitochondrial, cytoplasmic and ER stress-related pathways which are interrelated and interdependent. Therefore, by decreasing calcium influx into the cytoplasm cancer cells can escape apoptosis. This can be attained by either downregulating the plasma membrane $Ca^{2+}$ permeable ion channels or by minimizing the efficacy of the signaling pathways that trigger these channels. The possibility of $Ca^{2+}$ overloading in response to pro-apoptotic stimuli is hampered by these protective actions and so

the effectiveness of mitochondrial and cytoplasmic apoptotic pathways is retarded. Yet another defence mechanism against apoptosis would involve cancer cell adaptation to the reduced basal [$Ca^{2+}$ ] ER without induction of pro-apoptotic ER stress response that usually accompanies ER luminal calcium imbalance (Prevarskaya et al., 2014) (Orrenius et al., 2003).

In full agreement with these general considerations, it was shown that PCa cells, upon transition to more aggressive androgen-independent phenotype, which is characterized by substantial enhancement of cell survival, down regulate their SOCE by decreasing the expression of the principal plasma membrane SOC-channel-forming subunit, ORAI1 protein as well as of the ER $Ca^{2+}$ sensor regulating SOC activation, STIM1 protein (figure 3) (Vandenberghe, Abeele, Roudbaraki, & Lepage, 2010). Furthermore, as androgen-response gene presents the ER luminal $Ca^{2+}$ binding protein calreticulin in the prostate, the $Ca^{2+}$ storage capacity of the ER is compromised by its lowered androgenin dependent PCa cells expression. It also actuates a chain of adaptive responses in the expression of other ER $Ca^{2+}$ handling proteins so that ER $Ca^{2+}$ filling stays at a lower level. The latter involves minimized SERCA2b expression to lower $Ca^{2+}$ uptake and elevates expression of ER-resident Bcl-2 protein which subsequently stimulate $Ca^{2+}$ leak from the ER (Abeele et al., 2002). Nevertheless, there are ways other than limiting $Ca^{2+}$ influx and maintaining low $[Ca^{2+}]_{ER}$, to alter the tools that can manage $Ca^{2+}$ to resist apoptosis in various cancer cell. There are numerous instances of higher expression $Ca^{2+}$ entry channels conferring apoptosis resistance (figure 3). Besides promoting proliferation, TRPV6 and ORAI3 also increase PCa and breast cancer cells survival. In addition to that, the breast cancer that survives due to ORAI3 is often implicated to the rise of c-Myc expression and function, which is reliant on $Ca^{2+}$. That leads to hinder the dissemination of pro-apoptotic Bax protein. Apoptosis resistance is conferred by higher levels of TRPA1 channel. It also demonstrates that, through

TRPA1-mediated $Ca^{2+}$ entry which leads to stimulation of ERK1/2 via Src, TRPA 1 channel encourages the small cell lung carcinoma (SCLC) cells to survive (Schaefer et al., 2013). Localized '$Ca^{2+}$ dependent anti-apoptotic signaling complexes' formation make the cancer cells capable to resist apoptosis and increase a certain type of plasma membrane $Ca^{2+}$ permeable channels. These channels contribute to increased amount of $Ca^{2+}$ (Prevarskaya et al., 2014). However, there are some other mechanisms by which $Ca^{2+}$ channels increase and lead to the prevention of apoptosis. These channels support $Ca^{2+}$ entry and carry on ER store refilling as a result it minimizes the actuation of apoptosis due to ER stress response which is regulated by $Ca^{2+}$ (figure 3). In addition to that, there are also positive correlation between the expression of the TRPV1 channel and grading of human glioma (astrocytoma). However, the apoptosis of glioma cells are triggered by exogenous TRPV1 agonists and endocannabinoids which is an endogenous agonists through ER-stress owing to TRPV1 localization in the ER membrane and not because of $Ca^{2+}$ entry via plasma membrane-localized TRPV1 (Stock et al., 2012) (Med, 2013).

Sequentially MPT and then the release of mitochondrial apoptosis-inducing factors can be triggered when mitochondria takes up $Ca^{2+}$stimulated by $Ca^{2+}$ entry or $IP_3R$-mediated surges of $[Ca^{2+}]_C$. Depending on the fact, the benefited $Ca^{2+}$ signal transmits from $IP_3R$ to mitochondria in the site where ER and mitochondria communicates, MPT associated with IP3R activation works. Therefore, $IP_3R$-mediated release which is compromised by down regulated expression or activation $IP_3R$ must be involved in the enhanced apoptosis resistance of cancer cells (figure 3). Moreover, it is shown that, in bladder cancer cells cisplatin-induced down regulation of $IP_3R1$ expression results in the acquisition of cisplatin resistance (Al-taweel, Varghese, Florea, & Büsselberg, 2014). Moreover, there are two usual protein Bcl-2 and $IP_3R$ that work against

apoptosis. They directly interact with one another for the inhibition of channel opening and the release of ER $Ca^{2+}$, which results in reduced apoptosis that is controlled by $Ca^{2+}$ (figure 3) (Preston et al., 1997) (Paj & Orzechowski, 2015).

PMCA2 and PMCA4 calcium pumps are expressed in breast cancer cells that work against apoptosis. Intriguingly, the inhibition of NFAT resulted from the direct interaction with calcineurin and via this PMCA2 induced apoptosis resistance. On the other hand, PMCA4 regulates the $NF_kB$ nuclear translocation similarly (Prevarskaya et al., 2014).

Autophagy is a process where the cell digests itself and elevates the survival of cancer cells. In this process the products inside the cells are reprocessed, cell synthesis and degradation are also well balanced. In severe cancer, they take part to pick cells that are highly tolerant to tumor-specific hypoxia and also unhealthy cells it leads to angiogenesis. It will probably make the cancer cells break away from the original tumor and resist the anti-cancer therapy (Article, 2015).

In the ER, there is a channel known as $IP_3R$ that secretes calcium and plays a very important role. In the ER membrane, $IP_3R$ is resided within a site where ER and mitochondria contacts. In addition to that, basal constitutive low-level $Ca^{2+}$ signaling is provided by $IP_3R$ that will sustain mitochondrial $Ca^{2+}$ uptake required for efficient mitochondrial respiration and maintenance of normal cell bioenergetics. This signal can be exterminated by blocking $IP_3Rs$ or suppressing IP3 production. Due to this, the formation of ATP will decrease and AMP/ATP proportion will be higher which will actuate AMPK and later on can cause autophagy. Therefore, when there is

interference in $IP_3R$-mediated ER–mitochondria in severe cancer associated with oxygen and nutrient shortage can actuate autophagy to make the cancer cells survive (Prevarskaya et al., 2014)

As increased adaption to ER stress, decreased MPT (mitochondrial permeability transition) and higher amount of $Ca^{2+}$ entry can lead to resist apoptosis in cancer cells, so targeting them could be effective against this apoptosis resistance (Decuypere, Bultynck, & Parys, 2011).

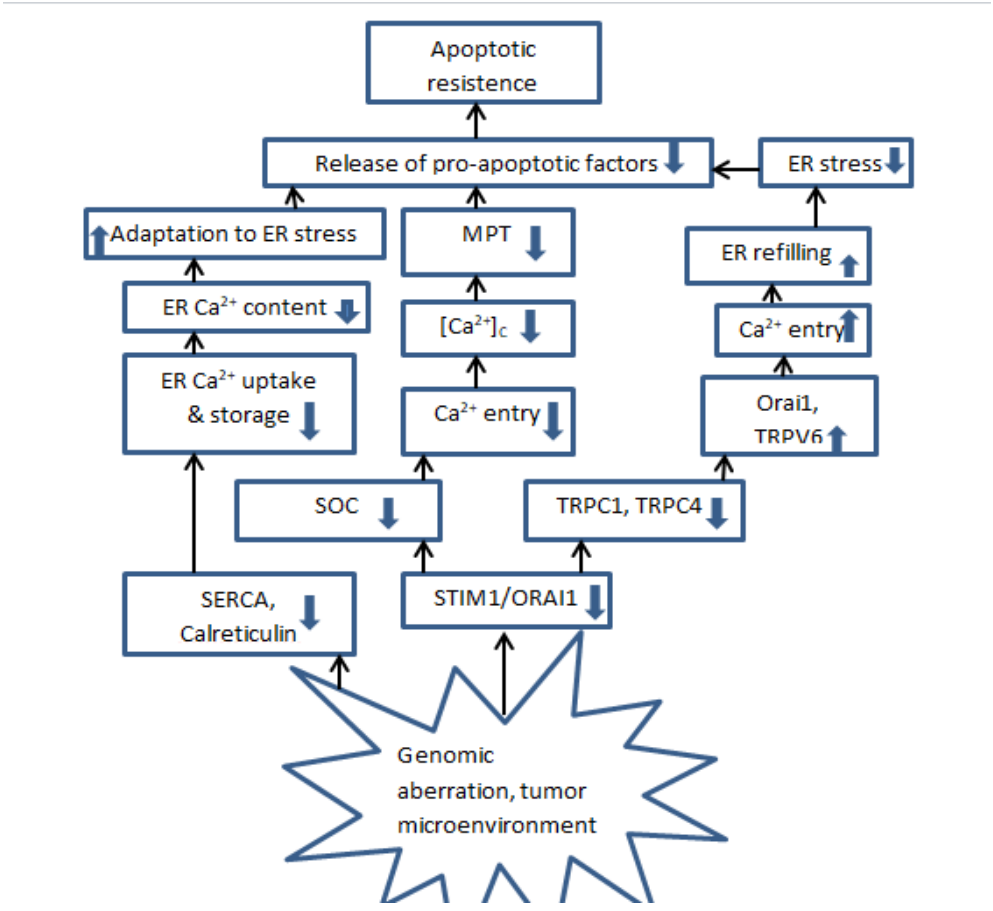


*Figure 3.$Ca^{2+}$ transport remodeling in conferring apoptosis resistance in cancer cells.*

Here, the upward arrows refer function or stimulation of some selected proteins or process. The downward arrows refer decreased expression of certain proteins or prevention of specified process.

## 2.4 $Ca^{2+}$ remodeling in tumor vascularization

For tumor growth and metastasis, vascularization is critical. Diverse extracellular signals can activate vascular endothelial cells and angiogenesis is controlled by cellular growth and motility. Tumor cells usually are able to release various types of peptides and growth elements. Among them endothelin-1, angiotensin II, basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) are the most commonly released ones that have the potentiality to stimulate mitosis or angiogenesis. From tumor and stroma cells, these factors release in a paracrine manner whereas for ECs they work only on the secretory cells. Angiogenesis and tumor progression are complex processes and in regulating the different critical phases of these processes intracellular $Ca^{2+}$ signals are involved. To induce $Ca^{2+}$ signaling which is involved in $Ca^{2+}$ entry and $IP_3R$ mediated release, VEGF acts directly on epithelial cells.

Carboxyamidotriazole is a compound that can work to prevent new blood vessel formation and tumor formation and administered by mouth. Additionally, these compounds have some characteristics that make them capable of targeting only the cancerous cells and not the normal cells. Moreover, this particular compound interrupts the conversion of signal to cellular activity that leads to preventing non voltage operated $Ca^{2+}$ channels. As a result, VEGF cannot be stimulated and cannot be released. This blocks the formation of new blood vessels. Therefore, $[Ca^{2+}]_C$ cannot be increased and the path to supply nutrients to the tumor cells are also blocked

(Lodola et al., 2012). This finally results in the prevention of tumor proliferation, severity and also the progression (Cui et al., 2017) (Florea, Splettstoesser, Dopp, Rettenmeier, & Dietrich, 2005).

Carboxyamidotriazole (CaI) works quite distinctly in case of SOCE channels and in EC it is made up of STIM1 and Orai1 proteins. When these proteins show irregularity in their activities it affects the $Ca^{2+}$ entry mediated by VEGF and also EC cell growth, survival etc. The formation of blood vessels are blocked as well (Cui et al., 2017).

Moreover, TRP family also has a part in the formation of new blood vessels (Ellis & Hicklin, 2008). These ensure $Ca^{2+}$ entry that are agonist mediated (figure 4). Specially TRPC1 and TRPC4 channels are responsible for the $Ca^{2+}$ influx and result in angiogenesis. In addition to that, TRPC3 or TRPC6 channels increase the vascular permeability by getting stimulated through VEGF. Moreover, STIM1, Orai1 protein also help angiogenesis and further lead to cancer cell growth, invasion etc. (Kwan, Huang, & Yao, 2007). Additionally, TRP family sometimes stimulates store independent $Ca^{2+}$ entry which increases the VEGF release that leads to increase ECs migration, survival etc. which finally results in tumor vascularization (Cui et al., 2017).

As VEGF release enhances the epithelial cells' development, migrating ability, survival rate as well as permeability, it results in tumor vascularization. So, preventing the increasing VEGF release could be a potential target to inhibit this type of vascularization.

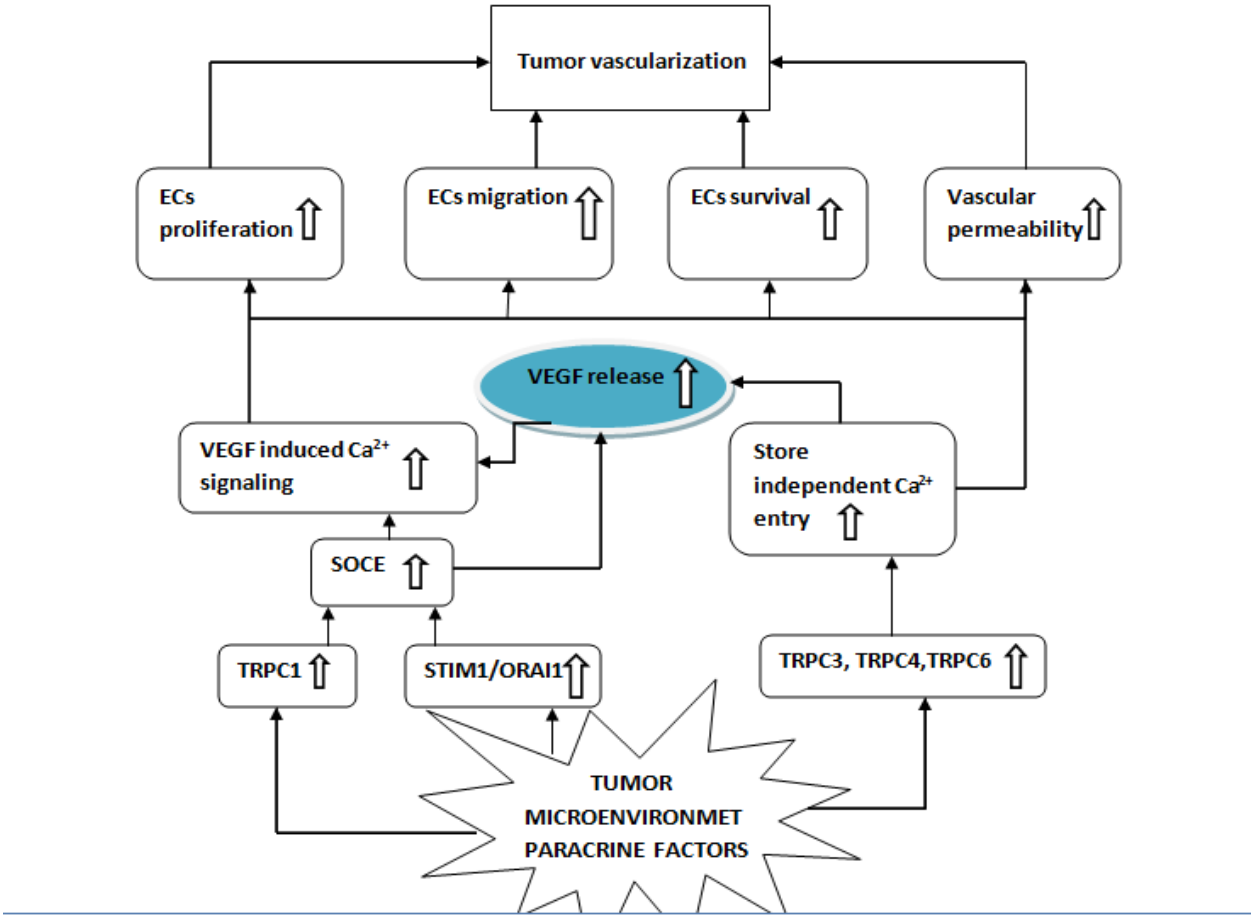


*Figure 4. $Ca^{2+}$ transport remodeling in tumor vascularization.*

This indicates event sequences step by step. The upward arrows show the increased expression of some certain proteins.

## 2.5 $Ca^{2+}$ remodeling in colorectal cancer

Various researches declare that, in colorectal cancer the VOCC is overexpressed. It is alarming that the mRNA of an isoform of the voltage-gated L-type $Ca^{2+}$ channel is elevated in colorectal cancer. However, this elevated mRNA that is correlated with the cardiac isoform of the L-type $Ca^{2+}$ channel is considered as a significant marker in colorectal cancer (Wang et al., 2000). Though the overexpression of VOCC has not been fully explored, α1 subunit protein is seen to be overexpressed throughout the colonic cell dividing period or in the non-confluent stage that results in cancer. When the scientists conducted some further research, it came to light that through acting on SOCE, salicylate can prevent CRC cell growth. Therefore, it can be said that SOCE has an important role in colorectal cancer. In addition to that, further researches claimed that intracellular $Ca^{2+}$ remodeling also has a role in CRC. The colorectal tumors are not properly differentiated and as a result, in developmental stage of some colorectal cancer, the plasma membrane $Ca^{2+}$ ATPase 4 (PMCA4) is down regulated (Villalobos et al., 2017). Therefore, errors in $Ca^{2+}$ efflux leads to cancer cell growth and prevent the cell from undergoing the apoptosis process and this is connected to remodeling of $Ca^{2+}$ signaling. It has been cleared from different studies that, the intracellular $Ca^{2+}$ concentration is higher in colorectal cancer when compared to normal colon cells. The increasing $Ca^{2+}$ influx can lead to higher $Ca^{2+}$ concentration. Therefore, plasma membrane resting voltage is likely to be much more on the negative side in CRC rather than normal colon cells. The cell growth in cancer cell is also much higher than normal cells. So it is evident that, the growth rate and SOCE are correlated. This demonstrates that, SOCE has a significant part in both normal and CRC cells. Moreover, a test has been conducted on HT29 cell and in that cell, preventing the SOCE resulted in inhibited CRC cell growth in the in vitro condition. In addition to that, elevated SOCE can also cause

cancer cell migration. For instance, ORAI1 protein and $K^+$ channel interact with $Ca^{2+}$ channel and this is capable of controlling the migration. The genesis of the channel complex is significant because the complete phenotype was not promoted by any of the individual proteins. So, ejecting this complex can be a potential target. When there is no complex channel, the $Ca^{2+}$ entry, cell migration and bone metastasis will be damaged. Therefore, all these information made it evident that SOCE can be an actual target for chemoprevention in CRC (Villalobos et al., 2017). G protein coupled receptor actuation makes the dissimilarities of SOCE more visible in normal and cancer cells. Moreover, $Ca^{2+}$ release which is triggered by agonists are seen to be lower in normal epithelium cells of colon compared to colon cancer cells. Though both agonists release $Ca^{2+}$ from intracellular stores, agonist-induced entry $Ca^{2+}$ occurs only in tumor cells. In addition to that, some studies and tests conducted on caged-IP3 compounds reported that intracellular stores in tumor cells secrete more $Ca^{2+}$ through IP3 than normal colon cells. However, it is contradictory with the fact that, ionomycin or cyclopiazonic acid trigger to secrete more $Ca^{2+}$ in normal cells rather than cancer cells (Villalobos et al., 2017) (Fuszek et al., 2004).

Though the agonist stimulated secretion of $Ca^{2+}$ are preserved in CRC cell, $Ca^{2+}$ reservoir is found to be vacant in CRC cells. $Ca^{2+}$ reservoirs are full but agonist stimulation causes the release of a small portion of reserved $Ca^{2+}$ in normal cells that cannot actuate SOCE. This is why, agonist stimulated secretion of $Ca^{2+}$ is normal in CRC cells (Villalobos et al., 2017). There are some other differences in storing $Ca^{2+}$ in normal and cancer cells. Stored $Ca^{2+}$ leads to apoptosis which occurs due to an overabundance of mitochondrial $Ca^{2+}$. Moreover, distinct renewal of the store encourages different types of cell death. This explains why $H_2O_2$ triggers

apoptosis in normal cells but not in CRC cells. Therefore, it leads to critical cancer hallmarks (Villalobos et al., 2017).

## 2.6 The molecular determinants of $Ca^{2+}$ homeostasis in prostate cancer cells

The epithelial cells in prostate cancer are not distinctive regarding the molecular determinants that take part in balancing $Ca^{2+}$ homeostasis. These cells are normally secretory types. Different cytosolic signals are formed from separate $Ca^{2+}$ reservoirs such as extracellular space, ER, mitochondria. They have their own separate membranes in which the proteins responsible for regulating the $Ca^{2+}$ are also present. These proteins help each of the reservoirs to generate $Ca^{2+}$ signals (Prevarskaya, Skryma, & Shuba, 2004) (Flourakis & Prevarskaya, 2009).

Through SOCE, the $Ca^{2+}$ enter into the epithelial cells of prostate cancer from extracellular space. When the ER content is reduced it allows influx of $Ca^{2+}$ through distinct plasma membrane or store operated $Ca^{2+}$ permeable channels (SOC) (Prevarskaya et al., 2004). Different studies and tests conducted on LNCaP human prostate cancer epithelial cell line, have showed the involvement of TRP subfamily TRPC1, TRPC4, TRPV6 in prostate specific endogenous SOCs (Prevarskaya et al., 2004). Among them TRPC6 is the most studied one as it is correlated to PC grades. Additionally, it is negatively dominated by AR. It was already known that AR is obstructed by anti-androgens that result in elevated amount of TRPC6 mRNA as well as store operated membrane current in LNCaP cells. Tissues from 140 people suffering from prostate cancer were collected and tested. The test results confirmed the correlation between PC development and TRPC6. Moreover, overabundance of TRPC6 increases $I_{SOC}$ membrane current that has specific characteristics. So, it becomes clearer that, TRPC6 activities in prostatic $I_{SOC}$ are interconnected with other SOC regulators (Prevarskaya et al., 2004).

In addition to that, a large amount of intracellular $Ca^{2+}$ is reserved in ER. $IP_3$, SERCA $Ca^{2+}$ pump, $Ca^{2+}$ binding proteins work as initial molecular determinants. There are various types of $Ca^{2+}$ transporters in mitochondria and through those they pass the $Ca^{2+}$ across their inner membrane. These mechanisms help to balance the $[Ca^{2+}]$ in local and global cytosol (Prevarskaya et al., 2004) (Haverstick, Heady, Macdonald, & Gray, 2000).

## 2.7 Remodeling of $Ca^{2+}$ influx pathways in breast cancer

$Ca^{2+}$ influx remodeling occurs in breast cancer cells and it is evident via the representation of different calcium channels influx pathways. The following $Ca^{2+}$ channels and/or $Ca^{2+}$ channel is seen to be changed in breast cancer cell lines and/or breast tumors. On the other hand, through pharmacological modulators $Ca^{2+}$ channels are connected to significant pathways in breast cancer cells (Jardin, Lopez, Salido, & Rosado, 2018).

### 2.7.1. Voltage-gated $Ca^{2+}$channels in breast cancer:

Among the $Ca^{2+}$ permeable ion channels, voltage gated $Ca^{2+}$ channels get very less importance in breast cancer. T type $Ca^{2+}$ channel blockers have much more potentiality to inhibit the proliferation of breast cancer cells (MCF-7) rather than a non-cancer derived breast cell line (MCF-10A) (Taylor et al., 2008). Breast cancer proliferation and T type $Ca^{2+}$ are associated with each other and result in high levels of mRNA for the $\alpha_{1G}$ and $\alpha_{1H}$ T type $Ca^{2+}$ channel sub-units in proliferating sub confluent MCF-7 and MDA-MB-231 breast cancer cells (Taylor et al., 2008). There is a T type $Ca^{2+}$ channel blocker named mifibredil that has the capability to inhibit MCF-7 breast cancer cell growth. Therefore, this supports the claim that, T type $Ca^{2+}$ channel blocker

has potential role in breast cancer treatment (Bertolesiet al., 2002). In addition to that, there are some metastatic breast cancer where the gene CACNA2D3 (α2δ3 subunit) is encoded in such a manner that the mRNA level of the voltage gated $Ca^{2+}$ channel subunit is decreased (Palmierietal., 2012). The role of down regulated CACNA2D3 in developing metastasis of breast cancer, is still unclear. In addition to that, any changes in CACNA2D3 level are not responsible for the metastasis (Azimi & Monteith, 2014). Nevertheless, the promotion of $Ca^{2+}$ homeostasis remodeling can be involved in the mechanism through other calcium transporters' compensatory up-regulation. Therefore, it results in increased migration or invasion capability and/or a modified sensitivity to apoptotic stimuli (Azimi & Monteith, 2014) (Warnier et al., 2015).

### 2.7.2. TRPC channels in breast cancer:

Among the different types of $Ca^{2+}$ permeable ion channels, TRP family is very important and well-studied in cancer treatments. It also plays an important role in case of breast cancer (Ouadid-Ahidouchet al., 2013). Many scientists have already assessed the expression of TRP channels in breast cancer. From these assessments, it was found that, if one breast cancer sample is compared with its paired non-tumor sample, there will be elevated amount of TRPC1 mRNA with a greater than 30-fold increase in 5 out of 10 human breast cancers (Dhennin-Duthilleet al., 2011). Yet, it is seen overexpressed mostly in small and low proliferation capacity tumors. That is why TRPC1 is not considered as a major target of advanced or aggressive breast cancers therapies (Dhennin-Duthilleet al., 2011). Apart from that, there are some other similar studies from where it is evident that, TRPC6 mRNA is also elevated in breast cancer biopsy samples compared with normal biopsy tissue in 7 out of 10 breast tumor samples (Aydaret al., 2009). In addition to that, from this study it was also reported that the growth of MDA-MB-231 breast

cancer cells can be hindered by TRPC6 silencing (Aydaret al., 2009). Apart from that, some breast cancer also shows an elevated amount of TRPC3 mRNA (Aydaret al., 2009). As TRPC3 has already been identified as a potential therapeutic target in ovarian cancer, it is also entitled for the further assessment in breast cancer (Yang et al., 2009) (Azimi & Monteith, 2014).

### 2.7.3. TRPM channels in breast cancer:

In some context of cancers like prostate cancer, the scientists have found out that the overexpression of the low temperature activates ion channel TRPM8. These studies have gained attention in case of prostate cancer and further encouraged the studies about overexpression of TRPM8 in breast cancer too (Tsavaleret al., 2001). However, the potentiality of TRPM8 in breast cancer cells as a therapeutic target has not been explored fully. But recent studies have revealed that, in oestrogen receptor positive breast cancers and well-differentiated lower grade breast cancers, TRPM8 overexpression is more common (Chodonet al., 2010). In addition to this, there is an isoform of the TRP family which is getting much attention in breast cancer therapies now a day. This unique isoform is TRPC7 and is permeable to both $Ca^{2+}$ and $Mg^{2+}$ and has its own kinase domain, for which some substrates have been identified (Runnels et al., 2001; Bates-Withers et al., 2011; Paraviciniet al., 2012). From the assessment of TRPM7 levels in human breast cancers it is found that, in higher grade and highly proliferative breast cancers TRPM7 is overexpressed. This plays a major role in proliferating MCF-7 breast cancer cells (Guilbertet al.,2009). Additionally, TRPM7 may be particularly important in breast cancer metastasis. Moreover, when the level of TRPM7 is increased in high-grade tumours, the survival rate drops drastically. This results in distant metastasis (Middelbeeket al., 2012). It is possible to deplete the cellular elongation and migration rate which are the key in vitro parameters of invasiveness in MDA-MB-231 breast cancer cellby the silencing of TRPM7. In addition to that, also in as in

vivo condition MDA-MB-231 breast cancer cells where TRPM7 has already been silenced have fewer metastatic potential (Middelbeeket al., 2012). Therefore, it is evident from the recent studies that, TRPM7 channel may be a potential pharmacological target for breast cancer therapy(Azimi & Monteith, 2014) (Lee, Monteith, & Roberts-thomson, 2006).

### 2.7.4. TRPV channels in breast cancer:

TRPV6 channel is the most important in breast cancer treatment and so it is much more proficiently studied. Similarly, to prostate, thyroid, colon and ovary cancer, breast cancer also has an elevated level of TRPV6 (Zhuanget al., 2002; Lehen'kyiet al., 2012). However, the level of TRPV6 is found to be up-regulated in some subset of breast cancer and from further studies it became clearer that the level of TRPV6 is elevated in a certain subset of breast cancer biopsies (Dhennin- Duthilleet al., 2011). Some scientists have reported from the recent studies that, the breast tumors in which the TRPV6 mRNA is elevated are usually oestrogen receptor negative and belong to the basal molecular subtype of breast cancer (Peters et al., 2012). However, the elevated TRPV6 mRNA associated breast cancer is less prone to survive. Therefore, silencing TRPV6 can be a treatment option for breast cancer. Silencing TRPV6 can lead to decreased growth of T-47D breast cancer cells (Peters et al., 2012). It also weakens the migratory and invasive features in MDA-MB-231 breast cancer cells (Dhennin-Duthilleet al., 2011). So, this can be used as a potential therapeutic target in breast cancer therapy.

Moreover, scientists also found out some connections of TRPV4 ion channels with breast cancer. Endothelial cell has been isolated from both normal breast tissue and breast cancer tissue and it has been found that TRPV4 level is higher in breast cancer tissues. Moreover, via arachidonic acid and 4α-phorbol 12,13-didecanoate it exhibits a greater $[Ca^{2+}]_{CYT}$ response to TRPV4

activation (Plaet al., 2012). These results establish, TRPV4 is a potential target in angiogenesis. Therefore, the migratory capacity of breast cancer derived endothelial cell is more developed rather than the normal ones through the activation of TRPV4. In addition to that, ‘arachidonic acid-stimulated migration’ of ‘breast cancer derived endothelial cells’ are reduced by TRPV4 silencing (Plaet al., 2012). For determining how the TRPV4 inhibition affects the inhibition of angiogenesis in breast cancer, compared to the clinically used vascular endothelial growth factor A inhibitors in vivo experiments are required (Azimi & Monteith, 2014).

### 2.7.5. Ligand-gated $Ca^{2+}$channels in breast cancer:

The researches on ligand gated $Ca^{2+}$channels in breast cancer are not good enough yet and need to be upgraded. For understanding some pathways and processes in breast cancer, ligand-activated $Ca^{2+}$channels are important. Recent studies gave some instances that P2X7 receptors are related to cancer cell invasiveness (Jelassiet al., 2011) and the anti-invasive properties ofthe anthraquinon eemodin (Jelassiet al., 2013). These receptors are evaluated by using a basal breast cancer cell, MDA-MB-435S which hasa strong melanoma-like feature. Basal and non-basal-like breast cancer cell lines studies and research should be improved so that the most therapeutically potent breast cancer subtype(s) and P2X receptor isoforms can be identified and metastasis can be controlled (Azimi & Monteith, 2014).

## 2.8 Drugs that target $Ca^{2+}$ channels/ transporters/pumps for cancer treatment

As the $Ca^{2+}$channels/transporters/pumps can be activated by different process, so some specific compounds can work on them and show chemotherapeutic activity. To develop these compounds

scientists must have a vast knowledge about the $Ca^{2+}$ signaling system inside the cell membrane. Additionally, these compounds should only target the cancerous cells and not the normal cells so that the aftereffects can be avoided. Scientists are trying hard to understand the structure and mechanisms of channels/transporters/pumps and conducting tests and clinical trials so that, their potentiality to be used as anticancer drug can be found out. (Cui et al., 2017) (Bose, Cie, & Wiechec, 2015)

### 2.8.1. Voltage-gated $Ca^{2+}$channel inhibitors

Usually the voltage gated $Ca^{2+}$ channel inhibitors are used in cardiac diseases. However, from various experiments the scientists came up with some evidence that they have significant roles in cancer too. Many analysts conducted various tests and experiments on VGCC inhibitors that are FDA approved and made it more evident. In the previous century, some VGCC inhibitors (L-type) which are not structurally related were tested to see if there is any effect of them in tumor growth. After so many experiments a dihydropyridine $Ca^{2+}$ blocker named amlodipine was discovered. It was tested on an A431 cancer cell both in 'in vivo' and 'in vitro' condition. This prevents cancer cell growth as it has the ability to halt the cell cycle at G1 stage. In addition to that, it lowers the level of cyclin D1 and kinase 4 which is regulated by cyclin(Cui et al., 2017). Moreover, there is another compound named mifibredil. It inhibits both T and L type channels and normally used to treat hypertension. However, it was retreated from the market because of the aftereffects which is suppressing P450enzymes, 2D6, 3A4 and P- glycoprotein. Due to these aftereffects it was remodeled to use in pancreatic cancer and high grade glioma therapy as it showed its ability to minimize the area of the tumor. Furthermore, from this, a new compound NNC55-0396 was discovered which can aim only the target and does not affect cytochrome

P450 3A4 in high extent. Therefore, this newly derived compound can be a potential drug in cancer treatment as it can prevent angiogenesis and it is also selective (Cui et al., 2017).

### 2.8.2. $Ca^{2+}$ ATPase inhibitor

The excess amount of $Ca^{2+}$ is lethal for cell as it can lead to cell death signaling. So, to ensure the apoptosis or necrosis of the cancer cell, the intracellular $Ca^{2+}$ can be increased by stopping these $Ca^{2+}$ ATP-ase pumps. In MCF-7 cells, these $Ca^{2+}$ ATP-ase inhibitors produce reactive oxygen species (ROS). They impair protein, lipid etc. and also stimulate $Ca^{2+}$ uplift. From previous studies it is reported that, reduction in endoplasmic $Ca^{2+}$ level leads to ER stress and causes apoptosis. So, targeting SERCA pumps and preventing them will result in reduction of ER $Ca^{2+}$ stores. For this purpose, thapsigargin (TG) can be a good option and it is also well studied in terms of prostate cancers. However, the problem is, thapsigargin does not work selectively on cancer cells only. It also affects the normal cells around the tumor by disrupting $Ca^{2+}$ balance. As a result, it causes some aftereffects. Keeping this problem in mind the investigators gave more concentration to target the specific site where the tumor is growing. To meet this criteria, the scientists have discovered a compound named G202 which was made by combining a derivative of TG and a peptide which only targets the prostate specific membrane antigen (PSMA) in prostate cancer. PSMA level can be seen higher in prostate cancer cells rather than the normal epithelial cells present in prostate. G202 is a prodrug and cannot work on non-cancerous cells. When G202 reach the target, the carboxypeptidase in PSMA breaks down the peptide and the TG derivative is discharged and shows its toxic activities on cancer cells. Later, this compound was named mipsagargin and was clinically tested on various cancer mostly on prostate cancer and glioblastoma(Cui et al., 2017) (Rodland, 2004).

### 2.8.3. TRP channel regulators

TRP channels can be targeted in cancer therapy as by blocking it, entry of $Ca^{2+}$ which are regulated by receptors can also be blocked. For this purpose, an imidazole compound SKF96365 is discovered that has the ability to minimize various functions of TRPCs. As a result, it prevents the proliferation of cancer cells in ovary. In addition to that, this compound lowers the level of TRPC6 in glioblastoma due to its high radio sensitive activities. Moreover, it is capable of halting the cell cycle at S and G stage in glioblastoma(Cui et al., 2017).

Moreover, TH117 was formed similarly as dihydropyridines so that it can stop TRPV channels. It has the capability to prevent the abnormal cell growth in PC. There are different types of TRPV channels. Among them TRPV6 is the mostly found one to support $Ca^{2+}$ entry in PC whereas TRPV is also common but 45 folds less than TRPV6. However, the problem is TH117 affects TRPV5 more rather than TRPV6. As a result, it cannot prevent much $Ca^{2+}$ entry and so it's not that effective to minimize cancer cell proliferation in prostate cancer and breast cancer. So this compound is still under examination and the scientists are working hard to make it selective towards TRPV6 (Cui et al., 2017).

There are some current compounds such as GSK1016790A which works as TRPV4 channel agonist. It is reported to be more potential than any other TRPV channels activator discovered before. As this channels have the capability to maintain angiogenesis and other functions as vessel maturation it can be used in combination therapy. Therefore, GSK1016790A is combined with cisplatin and used in treating cancer so that it can perforate the tumor. There are some other TRPV6 channel inhibitors which are still under clinical examinations. Among them SOR-C13,

SOR-C27 are mostly known and work similarly as C- terminus of soricidin. These compounds are mostly used in ovarian cancer (Cui et al., 2017).

Furthermore, a compound called capsaicin has the capacity to stimulate cell death in TRPC6 but does not have any effect on TRPV1. Moreover, there are some other compounds as capsazepine (CPZ) which is able to prevent the proliferation of oral squamous cell carcinoma in vivo. These compounds produce reactive oxygen species that lead to cell death but this is also independent of TRPV1 (Cui et al., 2017).

On the other hand, sequesterpene (-) enlergine actuates TRPC4 and TRPC5 that lead to huge $Ca^{2+}$entry and results in cell death. Though the aftereffects are a matter of concern and this compound needs to be modified (Cui et al., 2017).

### 2.8.4. Orai inhibitors

Orai inhibitors normally target the CRAC or SOCE. Calcium release activated channels help to refill $Ca^{2+}$ in the ER store that lead to the proliferation of cancer cells. Therefore, blocking these channels can be a potential target for cancer therapy. These channels can be blocked by trivalent ions $La^{3+}$ and $Gd^{3+}$ as they are capable of obstructing CRAC opening which was generated by I-II loop region of Orai1. Apart from this, they can also block other $Ca^{2+}$ channels (Cui et al., 2017).

Among different Orai inhibitors SKF96365 is mostly studied. This compound is able to prevent the cancerous cell proliferation and distribution in vivo. In addition to that, based on a test conducted on nude mice it is reported that, these compounds are capable of preventing SOCE in esophageal cancer (Cui et al., 2017).

Moreover, 2APB which is a SOCE inhibitor is also used in cancer treatment. This compound has the ability to prevent Orai1. In addition to that, it does not disrupt the interactivity of STIM and Orai1. However, it shows the aftereffects and not used as a potential drug as it is non selective. Various attempts are taken to modify this compound so that it can work selectively and prevent cancer cell growth in different types of cancer cells (Cui et al., 2017).
In addition to that, there are some other Orai inhibitors. One of them is RO2959 which is also a SOCE inhibitor. This has the ability to prevent a large amount of cell activities that depend on $Ca^{2+}$ such as generation of cytokinin, gene expression, T cell formation etc. However, the problem is the cells need to be early incubated for half an hour and due to this it does not work directly on Orai1 channel (Cui et al., 2017).

### 2.8.5. Miscellaneous

Apart from the mentioned ones there are some more compounds that work on $Ca^{2+}$ channels/ transporters etc. Among them the most popular one is xestospongin B which has the capability to block $IP_3R$. As a result, cancerous cell growth and metastasis is automatically inhibited and leads to programmed cell death in breast cancer. Moreover, a RyR agonist, caffeine helps to stimulate cell death in breast cancer whereas 4-choloro-m-cresol prevents cancerous cell proliferation. In addition to that, OSW-1 causes cell death by overburdening the mitochondria with $Ca^{2+}$ in leukemia. Brilliant blue G, Emodin etc. are some other compounds that increase cancerous cell death (Cui et al., 2017).

***Table 1:*** *List of compounds targeting $Ca^{2+}$ channels / transporters / pumps*

| Target | | Compound | Mechanism | Cancer |
|---|---|---|---|---|
| $Ca^{2+}$ ATPase | SERCA | Cyclopiazonic acid, thapsigargin, G202, KP1019 Saikosaponin-d, Alisol B | Inhibitor | Prostate, Hepatoma, Colon, Cervical, Breast Cancer |
| | SERCA2 | RL71 | Inhibitor | Colon Cancer |
| | PM CA | Pt(O,O0-acac)(γ-acac)(DMS)] | Inhibitor | Breast Cancer |
| VGCC | Ttype | KYS05047 ,mibefradil, NNC- 55- 0396, amlodipine | Blocker | Hepatoma, Lung Pancreatic Cancer, Epidermoid Carcinoma And Glioma |
| | | Ghrelin | Increase Protein Expression | Prostate Cancer |
| TRP | TRPA1 | HC- 030 031 | Inhibitor | - |

| | | | | |
|---|---|---|---|---|
| | | Polygodial and analog | Activator | Glioma, Melanoma, Uterine, Lung Breast Cancer |
| | TRPC | 20- GPP D | Activator | Colon Cancer |
| | TRPC | SKF963 65, M80 4 | Blocker | Glioma |
| | TRPC1 | EVP45 93 | Inhibitor | Neuroblastoma |
| | TRPC4/5 | (_)-EnglerinA | Activator | Renal And Colon Cancer |
| | TRPC4/5 | M804 analog, ML204 | Inhibitor | - |
| | TRPC3/6 | GSK23322 55B ,GSK28335 03A | Inhibitor | - |
| | TRPC6 | GaQ3 | Induce Protein Expression | Breast, Lung, Hepatoma |
| | TRPV | CPZ | Inhibitor | Oscc |
| | TRPV1 | CBD, Capsaicin | Agonist | Colon Cancer, Renal Carcinoma . |

| | | | | |
|---|---|---|---|---|
| | TRPV2 | 2- APB , cannabinoid, lysophospholipid and probenecid .<br><br>Ruthenium red ,TEA, TRI M, 4- amino pyridine, SKF 96365 | Agonist<br><br>Antagonist | Bladder Cancer<br><br>Breast Cancer |
| | TRPV4 | GSK10167 90A.<br><br>GSK21938 74, RN- 989 3, BTP2 | Agonist<br><br>Inhibitor | Prostate Cancer<br><br>- |
| | TRPM8 | CBG, M8- B<br><br>D- 3263 | Inhibitor<br><br>Agonist | Lymphoma, Lung, Breast, Prostate And Skin Pancreatic,<br><br>Various Advanced Cancer |
| | TRP ML | ML-SA1 | Agonist | - |
| | TRPML1 | MK6- 83 | Agonist | - |
| | TRP V6 | TH- 117 7, Soricidin, SORC13 and SOR -C27 | Inhibitor | Ovarian, |

| | | | | |
|---|---|---|---|---|
| | | | | Prostate And Brain Cancer |
| Orai | CRAC | Carboxyamidotriazole, dihydropyridine, MR S- 1844, MRS- 1845 ,BTP2 | Inhibitor | Hepatoma, Lung, Bladder, Kidney, NSCLC, Glioma And Leukemia |
| | STIM 1 | ML- 9 | Translocatio n Inhibitor | Prostate Cancer |
| | Orai1- STIM 1 | SKF963 65 | Inhibitor | Breast And Colon Cancer |
| | Orai1 | La3þ, Gd3þ, AnCoA4,SB01990,SPB06836,KM06293,RH0 1882,GSK- 5503A,GSK-7975A,mAbs | Inhibitor | Lung Cancer And Glioma |
| | - | 2- APB and its analogues, DPB- 162AE and DPB- 163AE | Inhibitor /Activator | Colon Cancer And Glioma |
| | - | RO2959 | Inhibitor | - |

## 2.9 Clinical prospects

There are many remarkable accomplishments in the understanding of remodeling $Ca^{2+}$ signaling to treat various types of cancer or to develop newer cancer therapy. Yet, these findings have a narrow practical utility for clinicians. As in the physiological activity of normal cells, the $Ca^{2+}$ handling toolkit has its involvement but targeting only the cancerous cell is a difficult matter. To minimize the invasive characteristics of cancer cells in 'in vitro' conditions and to prevent the development of neoplasm in 'in vivo' animal models, $Ca^{2+}$ transport proteins are targeted and for this purpose various technologies are used. However, these technologies are not fully developed and also have some failings in them.

In addition to that, in case of high-grade astrocytoma synthetic vanilloids are recommended as therapeutics now a day. The TRPV1 channel which is present in ER is highly elevated in this type of cancer cells and by activating this ER-stressis induced that results in tumor cell death. When the $Ca^{2+}$ is overburdened in the cell for a prolonged time or the $Ca^{2+}$ starts to deplete in ER, it can lead to programmed cell death. Therefore, it can be considered as a potential method in cancer therapy. From the plant-derived sesquiterpene lactones and their derivatives, SERCA pump inhibitors are used and they block ER $Ca^{2+}$ uptake that result in $Ca^{2+}$ dependent apoptosis and this is one of the realizations of this approach. These compounds can be lethal to the non-cancerous cells as well if they are administered systemically. So it can be said that they should be given as a combined therapy with other compounds such as prostate specific antigen in terms of prostate cancer (Prevarskaya et al., 2014).

## 2.10 $Ca^{2+}$ signaling and cancer: new horizon

In the last decade, vital advancement has occurred in our conception of redesigning the calcium signal and these calcium channels or pumps work as a potential target for cancer therapy. However, in some research areas like “emerging hallmarks of cancer” calcium signaling is still relatively unexplored. This also involves transformation of the energy metabolism to glycolysis. Possible role of $Ca^{2+}$ signaling in regulating glycolysis, the switching of glycolysis, and the use of glycolysis-generated ATP to fuel $Ca^{2+}$ pumps in cancer cells are required to be studied further for more development. The next decade will be more advanced in cancer biology. The scientists are trying to focus on tumor microenvironment where $Ca^{2+}$ signaling is very compelling. Moreover, the importance of platelet derived growth factor (PDGF) in the signaling between cervical cancer cells and cancer-associated fibroblasts and the ability of platelet derived growth factor (PDGF) to elevate cytosolic [$Ca^{2+}$] in other cell types reflect that $Ca^{2+}$ may be critical to this signaling. Hopefully, in the further years the world will be able to see the undetected roles of $Ca^{2+}$ in cancer as well as the newer compounds that specifically target $Ca^{2+}$ channels or pumps. In addition to that there will be more selective drugs free from adverse effects and cancer treatment will be more developed.

# Chapter 3

## Future Studies

For now, only a few drugs' in vivo and in vitro study were done. In the future there is scope for researching and doing the in vivo and in vitro test for some other cancer drugs as well. Moreover, there is opportunity to increase the potentiality of the existing drugs. Targeting calcium homeostasis is mostly done in breast cancer, prostate cancer and colon cancer but future studies can be done on several other types of cancer such as glioblastoma etc. to see how they affect the cancer cells.

# Chapter 4

# Conclusion

Therefore, to sum up we can say that, from this review, it is clear that $Ca^{2+}$ channels/ transporters/ pumps can be targeted in different types of cancers. The dysregulation of $Ca^{2+}$ homeostasis may work as a 'driver' rather than a 'passenger' in carcinogenesis or tumorigenesis. There is an emerging area of identifying and developing new chemotherapeutic agents that will help in cancer treatment and some of them are being studied in clinical trials. The importance of $Ca^{2+}$ channels/transporters/pumps in natural cells and their activities is huge. By keeping this in mind, the development of new cancer drugs should be done in such a way that it only targets the $Ca^{2+}$ signaling proteins through identifying their special characteristics. As a result, the normal cells will not be affected and there will be no adverse effects. Furthermore, recently the scientists are concentrating more on identifying the $Ca^{2+}$ channels that help directly in cancer cell growth. For the upcoming years the spotlight will be on designing such drugs that will be more distinct, structurally more effective and safe to treat the cancer patients. So targeting calcium homeostasis in cancer treatments should be more studied and developed as it has vast opportunities.

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