

# Review the Regulation of Plasma Membrane Calcium Channel in Cancer and Patch Clamp Technique

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**Abstract.** As one of the most versatile and universal second messengers, calcium plays an essential role in cell life. Here we briefly reviewed the research progress of how different calcium channels are located at the cell plasma membrane, including voltage-gated calcium channels (VGCCs), receptor-operated channels (ROC), and store-operated channels (ROC). These channels can regulate different cancer progression. Afterward, the patch clamp technique's development and operating principle, an important quantitative method used for ion channel investigation, are introduced in this paper.

## 1 Introduction

Calcium ( $\text{Ca}^{2+}$ ), known as a versatile and universal second messenger, is selected by a long course of organic evolution. It can transduce extracellular signaling and intracellular signaling to regulate numerous enzymes and proteins and further contribute to a wide range of cellular activity. Such universal cellular activities include fertilization, embryonic development, differentiation, transcription factor regulation, apoptosis, autophagy, cancer, and even virus infection [1-4]. The uniqueness of  $\text{Ca}^{2+}$  signaling is determined by its unique concentration gradient distribution among extracellular, cytosolic, and the endoplasmic reticulum (ER) in resting state. This feature gives  $\text{Ca}^{2+}$  the ability to regulate cellular processes in diverse spatio-temporal patterning, amplitude, and waves [1]. The history of scientists targeting calcium channels for disease treatment can be traced back to the 1960s, when the therapeutic effect of dihydropyridine molecules in cardiovascular disease was found [5]. In the last half century, great efforts have been put into studying how to target calcium channels for clinical therapy. Recently, more and more evidence suggest the calcium channels would be potential drug targets for cancer therapy, and  $\text{Ca}^{2+}$  signaling regulates cancer development in several aspects. Moreover, several  $\text{Ca}^{2+}$  channel inhibitors can efficaciously restrain the cell growth in human melanoma, colon carcinoma, prostate cancer, *etc.* [6,7]. Here, we intend to focus on the latest research advances about  $\text{Ca}^{2+}$  signaling regulation in cancer and reveal the mechanism of those regulations as well as patch clamp technique, the most common quantitative detection method for  $\text{Ca}^{2+}$ .

## 2 Role of $\text{Ca}^{2+}$ Channels in Cancer

Tumorigenesis is caused by mutation whereby normal cells are endowed with cancer-specific hallmarks, including self-sufficiency in growth signals, insensitivity to anti-growth signals, and evading apoptosis, *etc.*, [8]. Since versatile  $\text{Ca}^{2+}$  signal precisely controls numerous cell activities with widely different spatial and temporal profiles [9], the function of  $\text{Ca}^{2+}$  channels in various tumor cells is also particularly important. The plasma membrane (PM) calcium channels/transporters serve as the outermost gates to maintain the cytosolic [ $\text{Ca}^{2+}$ ] rapidly by controlling the influx of extracellular  $\text{Ca}^{2+}$  or extruding the intercellular  $\text{Ca}^{2+}$ , thus keeping the homeostasis together with those intercellular calcium channels. So, here comes a significant question: how plasma membrane  $\text{Ca}^{2+}$  channels contribute to the intercellular calcium homeostasis, and how broken homeostasis led to cancer development. It is acknowledged that cell proliferation is closely related to the cell cycle. The cell cycle contains four primary phases, which are G1 phase, S phase (DNA synthesis), G2 phase, and M phase (or mitosis). The checkpoint between each phase strictly controls the phase transition, and it was reported that calcium signaling participates in each phase and the cell circle checkpoint. The dysfunction of upstream PM  $\text{Ca}^{2+}$  signaling pathways can affect the downstream signaling pathways and change normal cell proliferation.

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### 3 Ca<sup>2+</sup> Channels on plasma membrane

The Voltage-gated calcium channels (VGCCs) are a group of channels mainly permeable to calcium ions in

#### 3.1 Voltage-gated Calcium Channels

**Table 1:** Different VOC  $\alpha 1$  Subunit Genes and Their Associated Cancer Types

VOC Type	Activated Voltage	$\alpha 1$ Subunit Gene	Significant Associated Cancer	References
L-Type	High Voltage Activated (HVA)	CACNA1S (Cav1.1)	Renal, Head-neck	[12] [13]
		CACNA1C (Cav1.2)	Brain, Lymphoma, Prostate, Renal	[14] [15] [16]
		CACNA1D (Cav1.3)	Lung, Renal	[17] [16]
		CACNA1F (Cav1.4)	Lymphoma	[15]
P-/Q-Type		CACNA1A (Cav2.1)	Brain, Esophagus, Breast	[18] [19] [20]
N-Type		CACNA1B (Cav2.2)	Brain, Male breast	[18] [21]
R-Type	Medium Voltage Activated	CACNA1E (Cav2.3)	Gastric, Prostate	[22]
T-Type	Low Voltage Activated (LVA)	CACNA1G (Cav3.1)	Ovarian	[23]
		CACNA1H (Cav3.2)	Brain, Bladder	[22] [24]
		CACNA1I (Cav3.3)	Brain, Breast	[22]

response to depolarized membrane potential [10]. Currently, there are five subtypes of VGCCs been recognized to include the L(Long-lasting)-type, N(Neural)-type, R(Residual)-type, T-type, and P(Purkinje)-/Q-type. Because the different subtypes of VGCCs take charge of different cell functions such as synapse transmission, mitotic signaling, and cell cycle progression [11], they synergistically contribute to cancer cells' proliferation. It is reported that the  $\alpha 1$  subunit of the protein constructs the ion-selective pore, and different VGCC subtypes contain  $\alpha 1$  subunit encoded by ten various genes in humans (as it shows in Table 1).

The function of low voltage-activated T-type VGCCs in cancer progression was studied at a very early stage. In the first place, people pay less attention to the role of VGCCs in breast cancer due to such cancer cells are non-excitabile cells in which the influence of VGCCs was less regarded [25]. Nowadays, it is acknowledged that VGCCs are expressed in various non-excitabile cells such as breast cells, kidney cells, and prostate cells. The overexpression of encoded gene CACNA1G was found to be capable of inhibiting proliferation in human breast cancer cell line [26], while block CACNA1H encoded Cav3.2 shows antiproliferation potential in breast cancer [27].

Recently, some revealed that the T-Type VGCC inhibitor such as mibefradil or TTL1177 could play an antiproliferation role in breast cancer by interfering with the G1/S checkpoint in the cell cycle [28]. It can also induce apoptosis through PI3K/AKT/mTOR pathway [29].

In the L-type (Cav1) family, most evidence indicates the mutation of the CACNA1D gene is responsible for prostate cancer and adrenal aldosterone-producing adenomas (APA) [30]. The significant over-expression of the CACNA1D gene is associated with the transactivation of androgen receptor and malignant prostate cancer [31]. The channel's abnormal expression exhibits in various cancers such as acute myeloid leukaemia and squamous cell carcinoma (CACNA1S overexpress). Meanwhile, it is downregulated in renal oncocytoma and squamous cell lung carcinoma (CACNA1S down-expression). Moreover, the downregulation of the CACNA1C gene in L-type is mainly responded to most brain tumour types [32].

The bioinformatic analysis also suggested that CACNA1A encoded P-/Q-type VGCC  $\alpha 1$  subunit specifically had low expression in colorectal, esophageal, and gastric cancers. Hence, the potential of P-/Q-type VGCC as a drug target for those cancers are worth further research [32].

#### 3.2 Receptor-operated Channels

Receptor-operated calcium channels (ROCC) are referred to as the non-voltage-gated Ca<sup>2+</sup> channels, which can be regulated by a series of G-protein-coupled receptors on the plasma membrane [33]. Recently, the role of these channels in cancer progression is increasingly appreciated.

P2 receptors are Ca<sup>2+</sup>, Na<sup>+</sup>, and K<sup>+</sup> permeable ion channels gated by ATP. This property endowed them with distinct functions in cancer development. Since

**Table 2:** Different TRP Channel Subtypes and Associated Cancer Types

Subfamily	Subtype	Cancer Type	References
TRPC (Canonical)	TRPC1	Glioma, lung carcinoma, Nasopharyngeal carcinoma	[49] [50] [51]
	TRPC3	Ovarian cancer	[52]
	TRPC5	Breast cancer	[53]
	TRPC6	Gastric cancer	[54]
TRPM (Melastatin)	TRPM1	Melanoma	[55]
	TRPM2	Prostate cancer	[55]
	TRPM4	Prostate cancer	[56]
	TRPM7	Breast, Lung, Pancreatic cancer, Nasopharyngeal carcinoma	[57] [45]
	TRPM8	Breast, Lung, Prostate cancer	[57]
TRPV (Vanilloid)	TRPV1		
	TRPV2	Breast cancer	[48]
	TRPV6		
	TRPV4	Gastric, Lung, <i>etc.</i>	[58] [59]

there is extracellular ATP (eATP) enrichment in the tumour microenvironment, Adinolfi et al. summarized that the P2 receptor subtype P2X receptor plays an important role in the differentiation, angiogenesis, and migration of tumour cells in 2015 [34]. Some then suggested that the activation of the P2X7 receptor could reduce the PI3K/Akt activity and participate in neuroblastoma progression [35]. It was also reported that the P2X receptor can act as Neoplastic ATP pipeline, thus refueling eATP into tumour interstitium [36]. Based on these findings, the P2X family could be a potential target for developing innovative cancer therapy.

The N-methyl-D-aspartate receptor (NMDA receptor) is another important ROC that contributes to intercellular Ca<sup>2+</sup> homeostasis. NMDA receptor is another drug target for cancer therapy because it regulates the mTOR signaling activity, hence modulate the metabolism, autophagy, and apoptosis of several cancer cell lines (e.g., gastric tumors, glioblastoma, small cell lung cancer, etc.) [37].

### 3.3 Store-operated Calcium Channels

As the VOC/ROC can be activated during the membrane depolarize in excitable cells, the Store-operated calcium channels (SOCC) regulate the Ca<sup>2+</sup> influx in various non-excitabile cells [38]. The store-operated Ca<sup>2+</sup> entry (SOCE) process is generally regulated by the intercellular calcium store, such as the endoplasmic reticulum (ER) and sarcoplasmic reticulum (SR). This activity can regulate the insensitivity to antiproliferation signals. So unsurprisingly, it was reported that among the SOCCs, several members in the transient receptor potential (TRP) channels superfamily are significant in cell proliferation and cancer progression [39] [40]. Another important SOCC network is the OARI-STIM

proteins, which are also reported to play crucial roles in cancer development.

It is acknowledged that the TRP superfamily is consist of 2 main groups and can be subdivided into nine subfamilies in animal, which are: TRPC, TRPN, TRPS, TRPM, TRPV, TRPVL, and TRPA in group one and TRPP, TRPML in group two according to the cladogram [41]. Normally, TRP channels respond to various stimuli like temperature and mechanical stress [42]., they are also reported to be influential to calcium homogeneous and cell cycle [22] [43]. So far, it is recognized that the TRPC, TRPM, and TRPV families are closely associated with cancer progression. As shown in Figure 2, the TRPC family reported being capable of presenting diagnostic markers in the progression of human ovarian cancer and nasopharyngeal carcinoma [44] [45]. Meanwhile, the TRPM family is identified to promote several cancer, especially prostate cancer [46] [47]. Moreover, the TRPM subfamily is identified to promote prostate cancer, pancreatic cancer, lung cancer, *etc.* Furthermore, convictive evidence suggests expression change of several members in the TRPV subfamily are associated with the progression of breast cancer [48].

There are also increasing studies targeting ORAI-STIM in the SOCC family as novel cancer therapy targets in recent years. ORAI1,3 and STIM1,2 (stromal interaction molecules) mediate the so-called store-operated calcium entry, and the structural biology of these two molecules determined their special operation mode. The STIM1 protein contains transmembrane domains include a canonical EF hand, a hidden EF hand, and the sterile alpha motif (SAM) domain inside the ER lumen. They operated as a calcium concentrate sensor [60]. Once the intracellular stores are depleted, the STIM1 molecules will oligomerize immediately and interact with ORAI to link the organelle and PM. ORAI will then inject calcium ions into corresponding

organelles [61]. In breast cancer, some suggested the ORAI-STIM mediated SOCE plays a crucial role in cell migration [62]. Moreover, the up regulation of ORAI and STIM also impact tumour growth and migration in several cancers, such as breast cancer, prostate cancer, colorectal cancer, cervical cancer, brain tumours, etc. [63].

## 4 Patch Clamp Technique the Essential Quantitative Methodology

The patch clamp technique, developed in the late 1970s, has become an indispensable tool in  $Ca^{2+}$  channel studies. In actual research work, it plays an important role in detecting calcium ion channels are antagonized or excited. It can accurately measure ion channel current. The patch clamp's basic principle is to isolate a single ion channel from the membrane by a micropipette and then measure the channel current. The key point is to form a high-resistance seal (up to 10 - 100 G $\Omega$ ) between the cell membrane and the micropipette [64]. Inside the micropipette are a single ion channel and an electrode so that the channel current will not leak outside but totally flow to the electrode. A tiny ionic current will be monitored by the electrode when ions pass through the ion channel. And then, the current will be amplified into a measurable value. In this section, the configurations of the conventional patch clamp are presented first. Then, some newly developed techniques for patch clamp are reviewed.

### 4.1 Conventional patch clamp

A conventional patch clamp system consists of a micropipette and an amplifier circuit. According to the attachment configuration between the micropipette tip and cell membrane, the patch clamp can be divided into four modes. The simplest mode is the cell-attached mode, as shown in Fig.1. The micropipette first attaches the cell membrane. Then a negative pressure is applied in the micropipette to form a G $\Omega$  seal. The single ion channel surrounded by the tip of the micropipette can be studied. However, the membrane potential is unknown and cannot be measured through this configuration. Based on cell-attached patch mode, if a higher negative pressure is applied so that the membrane under the pipette tip is broken, the cytoplasm will be directly contacted with the pipette solution. This mode is called whole-cell mode. Under this mode, the total currents of all ion channels can be directly measured and recorded.

In addition, there are two cell-free modes called inside-out excised patch and outside-out excised patch, as shown in Fig.2. The inside-out patch is obtained by pulling away the micropipette from a cell-attached configuration. Similarly, the outside-out patch is obtained by pulling away the micropipette from a whole-cell configuration. Table 3 summarizes the advantages and disadvantages of these two configurations.

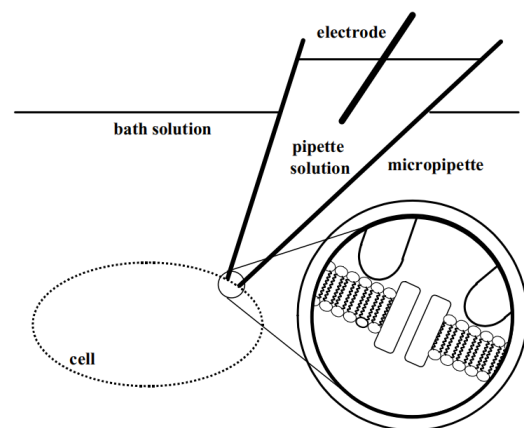


Fig 1. Cell-attached mode configuration [65]

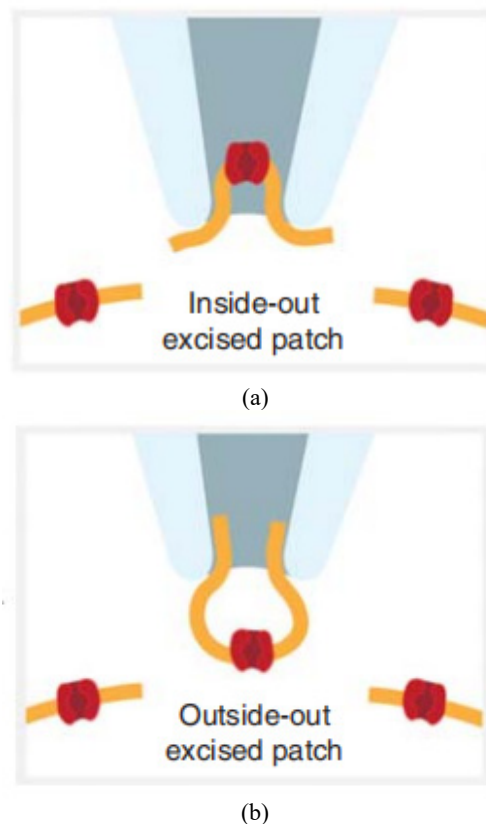


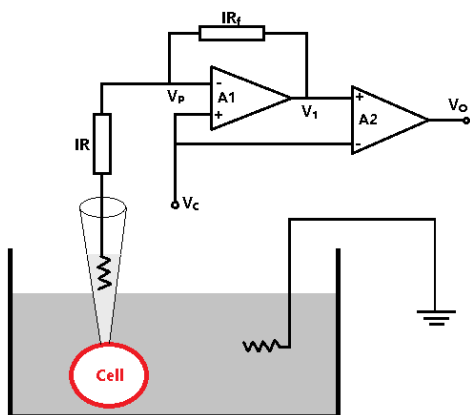
Fig 2. Cell-free modes configurations, (a) Inside-out, (b) Outside-out [66]

To study the ion channel behaviour and properties in detail, quantitative researches are necessary. According to the electric circuit's configuration and the working principle, patch clamp can be classified into two different types: voltage clamp and current clamp. Voltage clamp can keep cell membrane potential unchanged while recording the membrane current. A constant current is injected through the pipette electrode for the current clamp, and the changing voltage is recorded. Voltage clamp is more widely used in  $Ca^{2+}$  channel studies. Fig. 3 shows the configuration of the voltage clamp. It always consists of a negative feedback amplifier (A1) and a differential amplifier (A2). Generally,  $R_f$  is extremely large and much larger than  $R$ , so  $V_O$  can be roughly equal to  $IR_f$ . It can be seen that

through the voltage clamp circuit, an extremely small membrane current is amplified to a measurable level.

**Table 3:** Summary of Inside-out and Outside-out Configurations

Configuration	Advantages	Disadvantages
Inside-out	Extracellular environment is controlled. Good way to study the effects of intracellular factors on ion channels.	Washout of cytosolic factors. Destruction of cell structure. Bath solution must be similar with the Cytosolic environment.
Outside-out	Cytosolic environment is controlled. Good way to study the effects of extracellular factors on ion channels.	Washout of cytosolic factors. Destruction of cell structure. Micropipette solution must be similar with the Cytosolic environment.

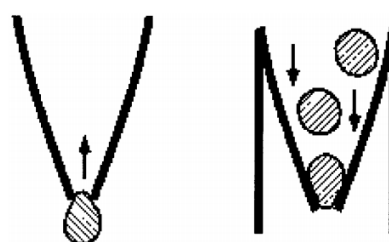


**Fig 3.** Voltage clamp configuration

#### 4.2 Automated patch clamp techniques

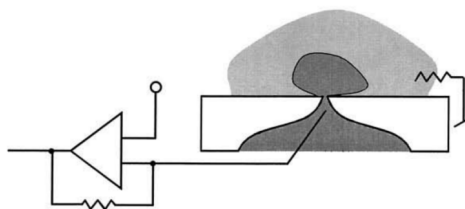
Conventional patch clamp techniques have some inevitable disadvantages [67] [68]. For example, the operations are time-consuming and very difficult. Operators need to micromanipulate a micropipette under a microscope to record the membrane current from one cell at a time. So that the throughput will be very low. What’s more, the intracellular solution cannot be changed easily during the experiments. Therefore, for some particular researches, the patch clamp has to work in many different modes, and a large number of experiments need to be done.

To overcome these disadvantages, scientists have developed automated patch clamp techniques. Lepple-Wienhues A, Ferlinz K, Seeger A, et al. (2003) [69] presented an automated patch clamp technology called “flip the tip”. In this method, the micropipette is filled with cell suspension. The suspended cells in the micropipette solution will be automatically flushed to the micropipette tip and form a high-resistance seal, as shown in Fig. 4. Compared with the conventional method, this method is much easier to operate. Moreover, a microscope and micromanipulator are no longer needed.



**Fig 4.** Conventional patch clamp (left) and “flip the tip” technology (right) [69]

Scientists also developed a planar patch clamp, which replaces the micropipette with a quartz chip with a small aperture. It was reported by Niels Fertig, Robert H. Blick and Jan C. Behrends (2002) [70]. Fig. 5 shows the configuration of the planar patch clamp. The cell suspension is given on the chip surface. The cell will automatically move to the aperture through an electric field or a negative pressure. Then, light suction is applied to absorb the cell and automatically form a high-resistance seal. Microscope and micromanipulator are also not needed in this method. Based on this planar patch clamp technique, Finkel et al. (2006) presented the population patch clamp technique [71], as shown in Fig. 6. The average current of multiple cells in a single well is recorded by a voltage-clamp. And the current in each well is measured at the same time. This method highly increases data consistency.



**Fig 5.** Planar patch clamp configuration [70]

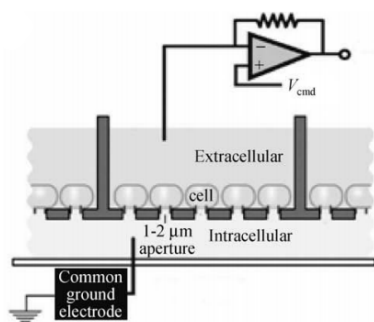


Fig 6. Population patch clamp configuration [71]

## 5 Conclusion

The regulation of calcium signaling is a super complicated system and has been studied by scholars all the time. In this paper, we generally reviewed the recent findings on several plasma membrane  $Ca^{2+}$  channels in cancer progression. With the support of more and more basic research and advanced bioinformatic techniques, the therapeutic potential of these  $Ca^{2+}$  channels has entered people's vision. However, many further investigations are required to verify whether these channels are ideal novel therapy targets in different cancer subdivisions. In addition, we also introduce the circuitry and principle of patch clamp techniques. The development of the automated patch clamp technique greatly liberated researchers' productivity, allowing them to invest more effort in targets identification.

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