Chapter 13

Ion Channels in Breast Cancer: From Signaling to Therapy

Dowd Jonathan, Hendin Josh,
Daniela F. Fukushiro-Lopes, David Laczynski and
Saverio Gentile

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Abstract

Breast cancer consists of an assortment of illness and therapeutic failure is mostly due to the complex and heterogeneous phenotype of the disease. Recently, changes in expression of several ion channels have been associated with malignancy including breast cancers. This suggests that breast cancer cells might gain a selective advantage by controlling ion channel expression/activity and that ion channels can contribute to the hallmarks of cancer. Due to the growing body of research demonstrating that ion channels are key factors in breast cancer biology. In this chapter, we discuss the role of specific ion channels in contributing to hallmarks of breast and whether these ion channels can be used as potential pharmacologic targets for breast cancer.

Keywords: breast cancer, ion channels, hallmarks of cancer, therapeutic targets

1. Introduction

Breast cancer is the most diagnosed cancer in women affecting more than 1.7 million women worldwide. Once metastasis has been detected, the average survival is 2 years and it is estimated that in 2016 about 250,000 women will be diagnosed with invasive breast cancer in the USA, and about 41,000 women under the age of 68 will die from the disease [1].

Molecular characterization of different breast cancer types offered the opportunity to separate breast cancers into two large groups that include luminal type [express estrogen receptors (ER); relatively good response to treatment] and the less common but more aggressive basal-like subtype (do not express ER; poor response to treatment). Innovations in targeted therapy are

being rapidly developed for the treatment of breast cancer, but the lack of suitable targets, limited drug availability, side effects, and drug resistance have severely hindered efforts toward improving outcomes in breast cancer patients [2].

Ion channels are pore-forming integral membrane proteins that create ionic concentration gradients by allowing flow of ions such as K^+, Ca^{2+}, Cl^- and Na^+ down their electrochemical gradients. There are at least 232 genes that encode for a variety of ion channel families that are organized according to ion channels function (IUPHAR: e.g., Kv potassium (K^+) voltage-gated) or gene name (HUGO: KCNH; K^+ voltage-gated channel subfamily H) [3, 4].

Variation in ionic gradients across cellular membranes plays a fundamental role in virtually all cellular events including electrical conductance, transcriptional regulation, contraction, secretion, motility, cell death, and proliferation [5, 6]. Activity of different families of ion channels can be gated by a variety of stimuli that range from changes in voltage (voltage-gated) and intracellular molecules to mechanical cues. In addition, ion channel activity can be modulated by a variety of events that are independent of their protein synthesis such as posttranslational modification (e.g., reversible phosphorylation) or epigenetically making ion channels one of the most abundant and functionally versatile classes of proteins. Therefore, ion channels are central in maintaining homeostasis and in pathological conditions.

Remarkably, recent research revealed that the expression level of several ion channels has been found altered in different types of breast cancers but not in healthy surrounding tissues [7–11]. Expression profiling of genes encoding for ion channels in breast cancers has provided evidence that the presence of specific ion channels can predict clinical outcome [12]. These studies indicate that changes in the activity of these proteins can potentially contribute to several of the hallmark of cancer and, therefore, to malignant transformation of breast cells.

2. Ion channels in cell proliferation

Cell proliferation is a complex, well-synchronized event that is stringently regulated by a number of ions, molecules, and proteins including K^+, Ca^{++}, ATP, cyclins, cyclin-dependent kinases, and many other cell cycle regulators that are associated with the cell-cycle machinery [13–19]. All cells present an intracellular negative electrical charge called transmembrane potential (V_m) that arises from the combined activities of a variety of ion channels/transporters, which create ionic gradients across the cell surface [20]. Transient decrease of this electrical charge (depolarization) followed by transient increase (repolarization) corresponds to key cell cycle checkpoints and it is critical for cell cycle progression of different cell types [21–27]. Several studies have established that in breast cancer cells, transient depolarization is a potent signal to initiate DNA synthesis causing ectopic reentry in the cell cycle, which is pivotal for malignant proliferation [22, 28]. In the MCF-7 breast cancer cell line, it has been observed that the Vm during a cell cycle progression correlates with the transition in each phase, such that, the pharmacological arrest of MCF-7 cells in G1/S or G2/M transition enriches cells with hyperpolarized Vm while cells arrested in the G0/G1 and M phases were enriched with depolarized Vm [10, 29].

Preservation of the oscillatory nature of membrane potential is necessary for cell proliferation. For example, chronic inhibition or chronic activation of a K^+ channel such as Kv11.1 produces persistent depolarization or hyperpolarization, which in either case can result in cell death or inhibition of proliferation (**Figure 1**) [30].

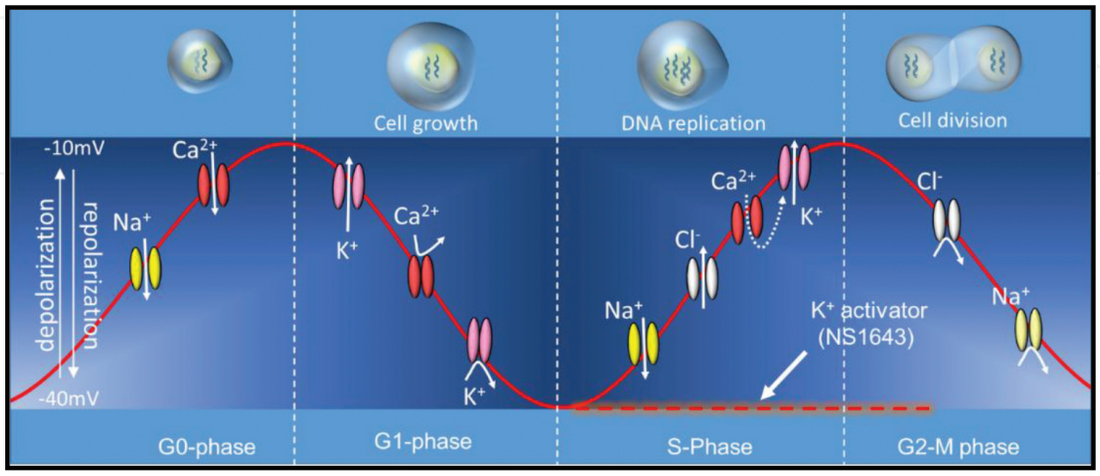

Figure 1. Schematic representation of ion channel activities during the cell cycle. Opening of the voltage-gated K^+ channels (e.g., Kv11.1) move positive charges from the intracellular to the extracellular space causing repolarization (red line). This event is required to promote transition from the G0/G1 to the S-phase of the cell cycle. In contrast, membrane potential during the S phase tends to depolarize due to opening of Na^+, some Ca^{2+}, and/or Na^+ channels. Mitosis is associated with more activity of Na^+ and/or Ca^{2+} that again depolarize the cell until duplication and return to repolarization in the G0/G1. Chronic application of a K^+ channel activator produces persistent repolarization. Conversely, K^+ blocker produces persistent depolarization. Both stop the cell cycle. ↓ = inward ionic flux; ↑ = outward ionic flux; ↺ = no ionic flux (Adapted from reference [10]).

3. Ion channels and the hallmark of breast cancer

Clinical differences of breast cancers are manifested by their histopathological characteristics, outcomes, and response to therapeutics. Nevertheless, the heterogeneity of breast cancers appears to be driven by the "classical" hallmarks of cancer identified by Hanahan and Weinberg which include: sustaining proliferative signaling, enabling replicative immortality, evading growth suppressors, resisting cell death, activating invasion and metastasis, evading immunodestruction, inducing angiogenesis, and reprogramming of energy [31–33].

3.1. Ion channels and proliferation of breast cancer cells

A growing body of experimental and clinical data supports the notion that ion channels can play a major role in contributing to these hallmarks in breast cancers [34].

It has been well established that a calcium ion is the universal signaling molecule in both physiological and pathological conditions [35, 36]. The intracellular concentration of calcium is kept at roughly 100 nM; however, cytoplasmic calcium can increase 100-fold upon specific cellular events. Calcium gradients are finely controlled by a sophisticated set of calcium permeant ions that are localized on the cell surface and intracellular membranes and can

regulate ionic fluxes from two major calcium stores: the extracellular space and the endoplasmic reticulum. Although calcium signaling plays a role in diverse cellular processes such as gene expression, cell growth, proliferation, apoptosis, migration, and among others, very little is still known about the role and functions of calcium channel in cancer biology.

The transient receptor potential (TRP) channels are a group of nonselective surface membrane cation channels that mediate a variety of sensations including taste, temperature, and taste [37, 38]. In addition, these channels can act as sensors for osmotic pressure, volume, stretch, or pressure.

TRPC6 (canonical) is elevated in breast carcinoma tissue compared to normal breast tissue and is functional, but it is not correlated with tumor grade, ER expression, or lymph node metastasis [39]. TRPV6 channel (activated by vanilloids and capsaicin) in breast cancer cells has been shown to provide cytoplasmic calcium necessary to promote downstream signaling for cell proliferation [40]. Pharmacologic inhibition of TRPV6 has been shows to sensitize breast cancer cells to apoptosis as well as decrease proliferation [41].

Furthermore, it has been found that activation of store-operated Ca entry (SOCE) in breast cancer cells leads to augmented expression of cyclins and suppresses cyclin-dependent kinase inhibitors, which ultimately leads to progression through the cell cycle.

Thus, abnormal expression of an ion channels family such as calcium channels in cancer cells could be considered as an adaptive mechanism by which the cells increase the frequency with which they proliferate [9].

K^+ is the most abundant intracellular ion and increased or decreased variation in [K^+] significantly contributes to changes of Vm during the cell cycle [10, 42]. Opening of K^+ channels allows K^+ to leave the cell resulting in depletion of positive charges from the cytoplasm, which contributes to repolarization. Temporary increased expression and/or activity of a K^+ channel drive a faster repolarization. This event can result in shortening the G1 phase of the cell cycle and increased proliferation [43].

Several voltage-gated K^+ channels (VGKC) such as Kv10.1, Kv11.1, and Kv1.3, the G-protein-coupled inwardly rectifying potassium channels (Kir3.1; GIRK1) or the two-pore potassium channel KCNK9 have been found to be overexpressed in different types of breast tumors suggesting that transcription of these K^+ channel genes is upregulated independently of the molecular characterization of breast cancers [12, 44, 45].

In contrast, other channels such as the potassium calcium-activated channel $K_{Ca}3.1$ have been found overexpressed mostly in high-grade breast tumors while an isoform of $K_{Ca}3.1$, $K_{Ca}1.1$ (or BK for short) has been found to be mostly expressed in tumors with lower grade [12]. Furthermore, breast cancer cells that metastasized to brain present higher expression of the BK channels compared to cells that metastasized in other body compartments [46, 47].

3.2. Control of ion channels activity in breast cancer

The expression level of potassium channels in breast cancers has been found to be controlled by a variety of factors, for example, mitogen-activated biochemical signaling. Estrogen can

control protein synthesis of several ion channels such as potassium channels [48], calcium channels, and sodium channels (via the novel membrane-bound G-protein-estrogen receptor (GPER) [49] or proteins that directly alter activity of ion channels such as the potassium channel tetramerization domain containing 11 (e.g., KCTD11) [50]. Furthermore, the β-adrenoreceptor (a G–protein-coupled receptor) can promote the growth of breast cancer cells by activating the GIRK potassium channel [51]. This indicates that these channel proteins might play a key role in sustaining proliferative signaling in luminal breast cancer cells.

The contribution of ion channel activity to proliferation can be finely controlled by a variety of cellular events including translational, reversible posttranslational, and epigenetic mechanisms. For example, it has been shown that the abundance of Kv11.1 mRNA encoded by the human ether-a-go-go-related gene 1 (hERG1) oscillates during the cell cycle and reaches its highest concentration in the G1 phase [52].

A timely increased expression of Kv11.1 translates into an increased exit of potassium ions from the cell which produces a faster repolarization. This event results in shortening the G1 to S transitions during the cell cycle and initiates a carcinogenic event [43]. Furthermore, it has been shown that hERG1 gene can undergo abnormal epigenetic regulation in breast cancer tumors which results in a considerably decrease Kv11.1 mRNA by gene promoter methylation [53]. Furthermore, mass spectrometry investigations revealed that Kv11.1 protein is among the 10 most phosphorylated proteins expressed in the breast tumors of MMTV-PyMT transgenic mouse [54]. Although the specific effect of this posttranslational modification has been characterized yet, it is well known that phosphorylation and dephosphorylation of Kv11.1 can drive dramatic changes in its activity [55–57] and it has been proposed that phosphorylated Kv11.1 channel might be the part of a not-yet-identified oncogenic signature [54].

Overall, these studies indicate that the contribution of ion channels to bypass the effect of growth suppression factors could be a consequence of a fine regulation of their activity via a reversible posttranslational and epigenetic mechanism.

4. Ion channels and apoptosis of breast cancer cells

Interestingly, ion channel activity has also been involved in suppressing proliferation by mediating apoptotic events or by activating a cellular senescence program in breast cancer cells.

Apoptosis is a cellular death mechanism controlled by a series of biochemical cascades that are activated by intrinsic (cellular stress) or extrinsic (signaling molecules from other cells) pathways. In both pathways, calcium is a necessary factor for the maintenance of the adequate signaling required for the effective execution of cell death [58, 59].

For example, it has been shown that the transient receptor potential-melastatin-like 7 (TRPM7) channels can be a target of caspase-8 and its cleavage mediates an inward calcium flux current during apoptosis [60]. In addition, suppression of TRPV6 functions by gene silencing reduces proliferation and activates apoptosis in breast cancer cells [41].

Furthermore, overexpression of the voltage-gated calcium channel $Ca_V3.1$ suppressed cell proliferation in luminal breast cancer cells while knockdown of the *CACNA1G* gene encoding for Cav3.1 promoted the cell proliferation. In contrast, overexpression of another member of the Cav3 family, $Ca_V3.2$, did not affect the cell proliferation [61, 62]. In their study, the authors showed convincing evidence of a differential distribution of $Ca_V3.1$ and $Ca_V3.2$ channels at plasma membranes of apoptotic and nonapoptotic cells, respectively.

In addition, the calcium-activated chloride channel CLCA2 has been found downregulated in breast cancers and it is considered as a candidate tumor suppressor [63].

Cellular senescence is characterized by a permanent arrest of the cell cycle without activation of cell death pathways. Senescence can arise as response to hyperactivity of oncogenes and it is considered an important tumor-suppressor mechanism [64–66]. Increased expression of the Kv1.1 potassium channel in human mammary epithelial cells appears to mediate oncogene-induced senescence while reduction of Kv1.1 protein level associates with augmented cancer aggressiveness [67]. Additionally, hyperactivity of the Kv11.1 channel produced cellular senescence in different human breast cancer cell lines independently of their molecular characterization [30, 68, 69]. Therefore, activation of Kv11.1 channel can reprogram breast cancer cells from replicative to nonreplicative immortality.

5. Ion channels in breast cancer metastasis

Metastasis is a multistep process in which cancer cells detach from the primary tumor and spread to other body compartments (secondary foci). The metastatic cascade can be summarized in three main steps including: (1) loss of cell-cell contact, (2) invasion of surrounding stroma and vasculature, and (3) extraversion into the tissue of the organ host. Activation of each steps of the metastatic cascade is controlled by a numerous signaling molecules including hormones such as epidermal growth factor (EGF) and transforming growth factor (TGFβ) [70]. Metastatic cells retain most of the hallmarks of cancer including proliferation, and they are able to form new tumors in distant parts of the body. Changes in expression and activity of ion channel proteins have been associated with each step of the metastatic phenotype.

5.1. Ion channels in loss of breast cancer cell-cell contact

It has been well established that cell-cell contact is guaranteed by a high expression level of adhesive molecules such as E-cadherin (epithelial cadherin) and loss of E-cadherin and/or increased vimentin expression promote the transitioning of cancer cells from an epithelial to mesenchymal phenotype (epithelial to mesenchymal transition; EMT) in which cells present an enhanced migratory behavior [70]. Changes in calcium dynamics play a major role in the EMT process as intracellular calcium chelation can strongly affect transcription of several EMT markers [71]. TRPM7 calcium channel mRNA is prognostic of disease recurrence and distant metastasis in breast cancer. In addition, suppression of TRPM7 activity inhibits the expression of EGF-dependent vimentin in metastatic breast cancer cells [71, 72], while TGFβ-dependent

EMT is directly associated with enhanced activity of two major components of the store-operated calcium entry channels, STIM1 and Orai1 [73].

In contrast, the activity of the chloride channel cystic fibrosis transmembrane conductance regulator (CFTR) plays a role in suppressing EMT in breast adenocarcinoma and metastatic cell lines. Suppression of CFTR is associated with reduction of E-cadherin protein level producing a weakened cell-cell contact [74].

5.2. Ion channels in breast cancer cell invasion

Invasion of cancer cells into surrounding tissues relies on the ability of cells to move through biological and physical barriers (e.g., extracellular matrix (ECM) and basement membranes). This process occurs by formation of membrane protrusions (e.g., lamellipodia and/or invadopodia/filopodia), which are driven by actin polymerization after cell polarization and formation of focal contact points between ECM and cytoskeleton. These processes are regulated by biochemical pathways that include a set of important proteins such as the focal adhesion kinase (Fak).

The increased expression level of TRPM7 in breast cancers correlates with metastatic phenotype [75, 76]. In ER-ductal adenocarcinomas, TRPM7 is increased in invasive cells and knockdown of TRPM7 impairs MDA-MB-231 cell migration *in vitro* and metastasis *in vivo* [77]. Interestingly, the TRPM7 contains both a calcium channel and a kinase. Rapid, local calcium permeability (calcium flickers) through TRPM7appears to play a role mostly at the leading lamella of migrating cells while its kinase activity has been directly involved in changing focal adhesion sites to generate the necessary driving force for movement [78].

Ectopic expression of the voltage-gated sodium channel Nav1.5 has been directly associated with the ability of breast cancer cells to migrate and invade surrounding organs [79]. Suppression of Nav1.5 activity by using blockers or siRNAs in breast cancers produced a strong inhibition of outgrowth/extension processes, migration, and invasion without affecting proliferation.

During the process of invadopodia formation, outgrowth is guaranteed by digestion of the surrounding ECM by secretion of cathepsins-like enzymes and metalloproteases such as MMP2 and MMP9. Activity of Nav1.5 has been correlated with increased cathepsin secretion [80]. Upregulation of both MMP2 and MMP9 enzymatic activity requires calcium [81]. Interestingly, in metastatic breast cancer cell lines, suppression of voltage-gated calcium channel activity inhibits MMP9 expression level [82] and stimulation of the purinoceptor calcium channel P2X7 (ATP-gated calcium channel) increases secretion of cathepsins and accelerates invasion [83].

Interestingly, analyses of the MMP23 enzyme (which is abundantly expressed in breast cancer cells) protein structure revealed the presence of a particular domain (TxD) that inhibits the activity of several voltage-gated potassium channels (Kv1.6, Kv1.3, Kv1.1, Kv3.2, and Kv1.4) by directly blocking ionic fluxes and inhibiting trafficking of these channels to the surface membrane of T cells [84, 85]. Activity of these channels in T cells is fundamental for proliferation as well as production of cytokines. Therefore, it has been proposed

that dual activity of MMP23 in breast cancer cells can favor invasion and suppress antitumor immunity.

5.3. Ion channels in breast cancer extravasation

In malignant cancer metastasis, extravasation refers to the ability of cancer cells to exit the capillaries and enter tissues. Typically, upregulation of the calcium channel transient receptor potential cation channel subfamily V member 4 (TRPV4) has been found strongly correlated with metastatic status of breast cancers [86]. Interestingly, increased activity of TRPV4 produced a softening of breast cancer cells and it has been associated with an extravasation trait in a murine breast cancer model, while suppression of the trpv4 gene significantly reduced lung metastasis.

6. Repurposing drugs targeting ion channels for breast cancer therapy

The body of research on the role of ion channels in breast cancer biology is growing and with the large availability of pharmacologic agents targeting the vast majority of ion channels, there is an interest in considering these proteins as potential novel therapeutic targets.

A study in which calcium channel blockers that have been already used in the clinic (e.g., antihypertensives such as verapamil) were tested for their effects on breast cancer biology showed that these compounds could increase the risk of intraductal and intralobular breast cancer. However, other studies showed no increased risk indicating that using these molecules as antibreast cancer agents is still debated [87, 88].

The nonvoltage-operated calcium channel blocker carboxyamidotriazole that is at this time in clinical trial shows antineoplastic potential [89] as it can produce decreased endothelial proliferation and angiogenesis in breast cancer cell lines.

More recently, the focus has moved to looking for agents that will specifically target the upregulated calcium channels seen in breast cancer cells. One such agent is lidocaine, a well-known anesthetic that inhibits sodium channels was found to reduce calcium influx through the TRPV6 channel and decrease the migration of breast cancer cells [90].

Several sodium channel blockers have revealed antitumor properties. Riluzole and carbamazepine, which are used for the treatment of neurodegenerative diseases, respectively, amyotrophic lateral sclerosis and epilepsy, have shown promising antitumor properties in metastatic breast cancer cells [91, 92]. Although the biochemical mechanism linking riluzole to inhibition of cell proliferation has been clarified yet, it has been established that inhibition of the sodium channel by carbamazepine produces an enhancement of proteasome-mediated degradation of ER alpha and human epidermal growth factor 2 (HER-2) [92, 93] indicating that these drugs might offer a therapeutic opportunity for both luminal and basal breast cancers. Furthermore, the anticonvulsant phenytoin can suppress migration and invasion of metastatic breast cancer cells [94].

Interestingly, some focus has moved from inhibiting the calcium channels to upregulating them with the idea being that the cancer cells, which already have more channels, become overwhelmed with the influx. Capsaicin was discovered to activate the TRPV1 channel in cancer cells. Administration of capsaicin was found to induce cell death in cancer cells [95].

7. Concluding remarks and perspectives

As ion channels play a fundamental role in virtually every cellular event, uncovering the contribution of these proteins in each of the hallmarks of breast cancer is important for understanding potential treatment for this heterogeneous collection of diseases. Ion channels are recognized as one the most important therapeutic targets and a large collection of molecules that can "correct" ion channel behavior have been traditionally employed to treat a vast variety of human diseases. Nevertheless, more research aiming to understand ion channel-dependent biochemical pathways, improve drug selectivity, and assess side effects is needed to convert promising discoveries on the use of molecules targeting ion channels as a therapeutic approach against breast cancer is needed.

Author details

Dowd Jonathan, Hendin Josh, Daniela F. Fukushiro-Lopes, David Laczynski and Saverio Gentile*

*Address all correspondence to: sagentile@luc.edu

Loyola University Chicago, Maywood, Illinois, USA

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