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Research Article

Role of Bioelectrical Signaling Networks in Tumor Growth

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Abstract

The ion channels are distributed in all cells and promote the rapid influx of ions that underlie the formation of cellular bioelectrical signals. Bioelectrical signals coupled with other regulator mechanisms provide fundamental physiological cellular processes, such as cellular differentiation, proliferation, and apoptosis, which are strongly associated with the manifestation of cancer hallmarks. Alterations in the bioelectrical signaling mechanism underlie the unusual bioelectrical features of cancer cells. Investigating the role of bioelectrical signals in tumor growth provides fundamental insights into cancer diagnosis and tumor-targeted treatment. Hence, this field of research is becoming one of the frontrunners of cancer medicine, and advances in biophysical tools are enabling progress in understanding this biological phenomenon. Recent studies have revealed that bioelectrical signals represent a promising target in cancer therapy. It is becoming increasingly convincing that cancer conditions can be reversed to normal by regulating the bioelectrical signaling mechanism of cells. Herein, we provide a brief review of the role of bioelectrical signals in cancer pathophysiology and provide data on the manipulation of this signaling mechanism as a novel approach to preventing malignant growth.

Keywords

Ion Channels, Bioelectrical Signals, Cancer Hallmarks, Spectroscopy, Electrophysiology

1. Introduction

Ion channels as molecular machines facilitate the passive movement of ions down an electrochemical gradient, produce ion currents, and provide the formation of bioelectrical signals. Recent advances in the structure-functional organization of ion channels and the biophysical characterization of ion current signals under physiologically relevant conditions provide valuable knowledge about cellular bioelectricity, which is an essential characteristic of a biological system [1]. Bioelectrical signals, as an integral part of cell signaling networks, play fundamental roles in crucial physiological processes [2-6]. Bioelectrical signal-mediated mechanisms represent a fascinating and flexible endogenous control system of the cells [7, 8]. It is widely known that the bioelectric properties of cancer cells differ from their normal counterparts, which implies that alterations in the bioelectrical signal function of the cells can promote the gain of hallmarks of cancer, uncontrolled proliferation, resistance to apoptosis, tissue invasion, and metastasis [9-15]. Recent studies continue to accumulate evidence that alterations of the ion channel properties linked to the generation of bioelectrical signals cause a reduction in the tumorigenic properties and cancer progression in cell lines and animal models [13, 16-18]. Thus, molecular-biophysical modulation of the bioelectric signaling may serve as a target to prevent or normalize tumors [19-22].

Numerous review papers have focused on the role of the ion channel and its produced ion currents on cancer hallmarks [10,

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14, 23-26]. This paper briefly reviews the role of major ion channel-driven bioelectrical signals in cancer and describes their manipulation as targets in cancer treatment. We also describe current biophysical technologies and tools for investigating the mechanisms of bioelectrical signaling.

2. Ion Channels Provide Bioelectrical Formation Signaling Network

Ion channels are pore-forming transmembrane proteins whose high selectivity allows the passage of inorganic ions, such as Ca^{2+}, Na^+, K^+, or Cl^- and provide a flux of ions down their electrochemical gradients into or out of the cell channels, which are found in all living cell membranes. The molecular biological, biochemical, electrophysiological, and genomic analyses allowed the identification of more than 250 ion channels from ~40 families [27].

The movement of several different ion species through various ion channels results in different electrostatic charges across the cell membrane. Sodium (Na^+) and chloride (Cl^-) ions are present at greater concentrations extracellularly than intracellularly, whereas potassium (K^+) ions are present at greater concentrations intracellularly than extracellularly. There are also organic anions, which are the most prevalent intracellular negatively charged molecules. The Na/K ATP-a pump plays a role in retaining the Na^+ and K^+ ion concentrations by actively transporting these ions against their concentration gradients. The Na^+/K^+-ATPase uses energy to transport ions against concentration gradients and pumps 3 sodium ions out of cells, while pumping two potassium ions into cells. The difference in electric potential between the interior and exterior of a biological cell is defined as membrane potential (*Vm)*, which is also known as transmembrane potential or membrane voltage. When the flow of positive and negative ions through channels is at the same rate (the cell is in a non-excited state), baseline *Vm* is called the resting membrane potential (RMP). In eukaryotes, RMP varies for different cell types, but is always negative (hyperpolarized). The typical RMP value varies between −10 to −100 mV. RMP acts as an energy source ("battery"), providing the cell with free energy that can be used for chemical and mechanical actions, which are essential for the survival of living species [19]. The action of ion channels promotes the formation of transmembrane ionic currents and causes changes in membrane potential. However, only certain ion channels are involved in the maintenance of the resting membrane potential and its alteration. These channels are highly selective for the three most abundant physiological ions, such as K^+, Na^+, and Cl^-, and play key roles in the formation of cellular bioelectrical signals. K^+ and Cl^- channels contribute to the maintenance of RMP, whereas the presence of Na^+ channels normally lead to a converse cellular excitability state [28].

Upon receiving a stimulus, the membrane potential of an excitable cell becomes depolarized at the threshold value of the membrane potential. This level of membrane potential allows the formation of an action potential (called "action potential", AP) when the voltage-gated Na^+ channels open and Na^+ ions flux inside. In the open state, Na^+ channels enhance the positivity of the intracellular membrane compared with the extracellular surface of the membrane. As the cell becomes more positive, voltage-gated K^+ channels open and K^+ ions diffuse out of the extracellular membrane (repolarization), resulting in activation of the Na^+/K^+ pump. The Na^+/K^+ pump bound three Na^+ ions from the inner side and two K^+ ions from the exterior of the membrane. In the end, the membrane potential returns to its normal resting state. This consistent, short-lasting fluctuation in the membrane voltage action potential (AP) is the definite basis of the membrane excitability [29].

The action potential is a major component of cellular bioelectrical signaling coupled with the mechanical contraction of a single cell and plays a leading role in the functions of the heart, brain, and skeletal muscle [30]. Accordingly, the bioelectrical signaling of cells is essential for the performance of the nervous system. In neurons, nerve impulses, or action potentials, propagate along axons or muscle fibers and constitute information that allows communication in the nervous system. During a nerve impulse, the membrane potential changes from its resting value of approximately 70 mV (negative inside the cell) to approximately +30 mV in less than 1 ms and returns to its resting value in a few milliseconds. Action potential in the cardiac system is responsible for cardiac muscle contraction. The right atrium of the heart, the sinoatrial node (SA node) generates about 60-100 action potentials per minute, resulting in an electrical impulse traveling through the heart's electrical conduction system to cause myocardial contraction. The cardiac action potential was approximately 300 ms, compared with 1 ms in the nerves.

The action potential during skeletal muscle initiation is a sequence of actions that results in muscle fiber contraction and relaxation. Skeletal muscle action potentials differ from action potentials found in neural and cardiac. In skeletal muscle cells, the action potential duration is approximately 2-5 ms.

The distribution of action potential and changes in the resting membrane potential (RMP) form a cellular bioelectrical signaling mechanism. The machinery of bioelectric circuits is coupled with biochemical and biomechanical cascades to establish cell behavior [2, 31, 32]. Recent structural-functional studies of ion channels provide an exciting insight into understanding how the bioelectrical signaling transduction mechanism regulates vital physiological events, such as proliferation, differentiation, cell shape, and apoptosis.

3. Transduction Mechanisms of Bioelectrical Signaling

a) Biophysical Tools for Structural Studies

Ion channels are remarkable transmembrane proteins that diffuse inorganic ions down their electrochemical gradients across cell membranes. Ion channels are complex biophysical machinery responsible for the generation of bioelectrical signaling circuits, and they provide the initiation of a wide variety of network interactions. Certainly, knowledge of the high-resolution structural organization of ion channels will offer a valuable framework for understanding the mechanisms of bioelectrical signaling. Recently advanced biophysical powerful techniques, such as high-resolution X-ray spectroscopy, have led to new insights into the structure of ion channel proteins.

X-ray crystallography is the most common technique for analyzing ion channel protein structures at atomic level resolution, providing an exciting view of the formation of bioelectrical signals. The first ion channel X-ray structure solved by MacKinnon's laboratory was the bacterial KcsA potassium channel [33]. This group has also determined the structures of numerous voltage-dependent potassium (Kv) channels, mechanosensitive channels [34-36], and the atomic structures of prokaryotic and eukaryotic members of the CLC chloride channel family [37]. Until now, many 3D structures of ion channels have been solved. Most of the structures of the ion channels were obtained using X-ray crystallography, which revealed the atomic based on structural organization of each superfamily of ion channels [38]. The voltage-gated ion channel superfamily usually has a tetrameric structure. Each of the four subunits (or repeats) comprises six transmembrane segments (S1–S6). There are 3 main structural parts: the voltage-sensing domain (VSD), which is formed by segments S1 to S4 and the connecting linker regions, the central ion-conducting pore domain (PD), which is formed by S5 and S6 of all four subunits, and the ion-selectivity filter, which is formed by the S5–S6-connecting pore loop when all four subunits come together [39]. The activation of ion channels (Kv, Nav, TRP, CNG, etc.) results from the voltage-dependent movement of charged residues in the voltage-sensing domain.

The cys-loop ion channel superfamily (nACh, 5-HT3, GABAA, and glycine receptors) has a pentameric structure [40]. The extracellular domain (ECD) contains the ligand-binding site. The transmembrane domain includes four alpha helices (M1–M4), and M3, and M4 helices span down to the intracellular domain (ICD), yielding a linker of 50–250 amino acid residues that participates in the assembly and trafficking of the cys-loop receptors [41].

Glutamate-gated ion channels (AMPA, NMDA, kainite receptors, etc.) are tetrameric ligand-gated ion channels with large extracellular domains, which are larger than those of other ion channels [42]. Glutamate receptors consist of the extracellular amino-terminal domain (ATD), extracellular ligand-binding domain (LBD), transmembrane domain (M1, M2, M3, M4), and intracellular carboxyl-terminal domain (ICD). Activation of glutamate ion channels is characterized by exclusive binding of the endogenous glutamate neurotransmitter, leading to opening of the pore region.

Acid-sensing ion channels (ASICs) belong to the trimeric proton-gated ion channel superfamily. They are characterized by a sizable extracellular domain (ECD), in which the acidic activation pocket resides, and two transmembrane domains (TM1 and TM2) for each of the three subunits, together forming the pore domain. Under acidic pH, a proton binds to the activation site in the ECD, leading to conformational changes in TM2, thus opening the pores of channels [43].

In recent years, cryo-electron microscopy (cryo-EM) has been one of the most widely used techniques to elucidate the structures of macromolecular complexes, specifically ion channels [44]. *Cryo-EM* has a few advantages over X-ray crystallography. In contrast to X-ray crystallography, Cryo-EM does not require the crystallization of molecules of interest, and proteins don't need to be highly purified. Instead, cryo-EM to study protein structure is frozen directly from the solution, making it possible to capture in an ion channel's near-native state and different conformational states. These structural alterations provide more insight into the conformational dynamics of ion channels [27].

Although protein size for *NMR spectroscopy* studies is limited (<~50 kDa), this powerful technique provides a great advantage in structural-functional studies. NMR spectroscopic techniques were successfully applied to probe the structural properties of ion channels close to physiologically relevant conditions [40, 41]. The first structure of a eukaryotic β-barrel membrane protein, a voltage-dependent anion channel from the mitochondria, VDAC-1, was solved using NMR spectroscopy [45]. NMR measurements revealed the binding sites of VDAC-1 for the Bcl-2 protein Bcl-xL, for β-NADH, and cholesterol, thereby providing an understanding of the mechanism by which VDAC contributes to mitochondrial homeostasis [46].

The most recent highly powerful electron paramagnetic resonance (EPR) spectroscopy in combination with the site-directed spin labeling (SDSL) method allows for the resolution of the conformational dynamics of proteins in a native environment [47]. The basic strategy of SDSL, which was pioneered by the Hubbell group, involves the introduction of a paramagnetic group at a selected protein site [48]. This process is usually accomplished by cysteine substitution mutagenesis, followed by covalent modification of the unique sulfhydryl group with a selective reagent bearing a nitroxide radical. SDSL EPR spectroscopy allows the determination of atom distances in the range of 1.8–8.0 nm, which is adopted for studying the number of ion channel protein architectures and motions. SDSL EPR spectroscopy allows us to reveal missing regions, and structural 3D validation provides a detailed view of the dynamic changes during activation [47].

Recently developed site-directed tryptophan fluorescence. *(SDTF)* was used to monitor the conformational dynamics of

proteins under physiologically relevant conditions [49]. Because information is obtained from intrinsic Trp fluorescence, unlike in the case of SDSL, this technique does not require additional labeling. The site-directed fluorescence technique has been applied successfully to explore structural information, conformational changes, hydration dynamics, and lipid-protein interactions of important classes of membrane proteins, such as ion channels/transporters [50].

b) Tools to Resolve Functional Bioelectrical Signals

During activation, ion channels undergo conformational rearrangements that provide the flux of ions, leading to the formation of bioelectrical signals. Although biophysical techniques, including X-ray crystallography, Cryo-EM, NMR, and EPR, provide high-resolution images of ion channel protein structures, this information represents isolated protein structures and conformational transitions. Electrophysiology techniques enable direct measurements of membrane currents in real-time and at the single-molecule level; a combination of structural studies and electrophysical methods can provide more insight into the bioelectrical signaling mechanism [39]. Patch clamping is one of the most general electrophysiological techniques used to study ion channel functions, and it could lead to potential breakthroughs in cancer research. The patch clamp method enables direct measurement of the membrane potential and/or the amount of current passing across the cell membrane at small ion currents of 10-12 A (pA) by clamping the voltage of an isolated excitable cell membrane [51]. There are several different patch-clamp configurations, which apply for studies and different purposes; (1) Cell-Attached: the cell remains intact and single channel currents can be recorded within the membrane patch, inside the electrode; (2) inside-out: the membrane patch off the cell (excised-patch configuration) exposes the cytoplasmic surface of the membrane for different additions, (3) whole cell: measuring the currents cross the plasma membrane of a single cell; (4) perforated: the technique allows electrical access between the cell and the patch pipette; and (5) outside-out: conventional whole-cell mode: the pipette can be pulled solely away from the whole cell, allowing a vesicle to be formed from the cell membrane. There are two ways for patch-clamp recording: voltage and current clamp. Under voltage clamping, the membrane potential was kept constant, and the membrane currents were recorded. In opposition, under the current clamp, the current through the membrane was held constant, and the membrane potential was recorded. In recent years, automated planar patch-clamp technology has been extensively used in academic research, as well as drug discovery, and it has been a remarkable breakthrough in the study of ion channels. Automated platforms allow the generation of high-quality information from studies of native and primary mammalian cells [52]. The development and application of one optical technique, voltage-clamp fluorometry (VCF), led to extending our knowledge about the structure of ion channels under physiological conditions [53-56]. VCF combines electrophysiology, molecular biology, chemistry, and fluorescence, which allows us to monitor the functional state and probe the structural rearrangements that occur as ion channels are activated by voltage. Voltage clamp fluorometry involves ion channel proteins that include a single reactive cysteine residue that are selectively labeled with an environmentally sensitive organic thiol-reactive fluorophore. Fluorescence changes can be monitored using the patch clamp technique, which represents voltage- or ligand-driven structural arrangements. Thus, ion channel currents are recorded related to certain conformational states, and this technique provides unexpected new insights into ion channel structure and function [55-58].

4. Bioelectrical Signaling Plays an Essential Role in the Development of Cancer Hallmarks

The activation of cell membrane ion channels is tightly regulated by their intrinsic gating mechanisms, which are, in turn, controlled by the presence of specific endogenous or exogenous physical or chemical factors. The ion channels generate electrical signals that are strongly coupled to intracellular biochemical signaling pathways. The interplay between these two mechanisms plays a critical role in fundamental cellular processes that are critical for cancer development and progression, including cell differentiation, proliferation, migration, and apoptosis [4, 14, 17, 59, 60, 73]. Any perturbations in the functions of this signaling mechanism could promote the development of certain pathophysiological conditions. Recent studies continue to accumulate evidence showing the importance of changes in ion channel-driven bioelectric signaling transduction pathways for the development of cancer hallmarks. Here, we focus on the role of bioelectrical signals in the formation of oncogenesis, which is produced by the ion currents of the major voltage-gated channels: sodium (Na^+), potassium (K^+), and chloride (Cl^-).

Voltage-dependent sodium channels (VGSC) are a multi-subunit, transmembrane glycoproteins. It is usually composed of an α subunit and one or more β subunits. The nine α subunits are termed Nav1.1–Nav1.9, and they are encoded by nine different genes (*SCN1A- SCN5A, and SCN8A to SCN11A*). NaV1.1, 1.2, 1.3, and 1.6 channels in the central nervous system, NaV1.7, 1.8, and 1.9 channels in the peripheral nervous system, NaV1.4 channels in the skeletal muscle, and NaV1.5 in the heart are primary cells [61, 62]. Nav1.5, 1.6, and 1.7 channels are also functionally expressed in several cancers, such as breast, colon, gastric, and lung cancers, as well as glioma and leukemia cells, and are involved in the invasion process and cancer progression [63, 64].

The first evidence of the presence of VGSC-mediated currents (I_{Na}) was reported in leukemic cancer cells, and the amplitude of (I_{Na}) increased in association with multidrug resistance in human leukemia cells. It was found that I_{Na}

represents an essential portion of cell bioelectrical value, which is exemplified by the high-level expression of VGSC in tumor cells [65, 66]. Most likely, I_{Na} regulates intracellular sodium homeostasis, which underlies its important role in the metastatic process of cancer cells [67]. It was suggested that the currents (I_{Na}) participated in the significant change in RMP in cancer cells between (-10 to -50 mV), compared to normal and non-proliferating cells (-50 to -90 mV). According to this view, the current range for VGSC-driven currents (I_{Na}) fits this RMP range, indicating that although the majority of VGSCs will be inactivated, only small VGSCs will increase the I_{Na}. Accordingly, increasing Na^+ concentration will promote increasing intracellular Ca^{2+} concentration, which will cause membrane depolarization and, consequently, cell migration and invasion.

Most investigations into metastasis have focused on Nav1.5, particularly for breast and colon cancer [68]. In breast cancers expresses a neonatal splice isoform of Nav1.5 rather than the adult isoform found in normal tissues [62]. It has been shown that pharmacological and transcriptional inhibition of SCN5A encoding the NaV1.5 channel leads to decreased cell invasiveness [69]. Furthermore, it was demonstrated that overexpression of the NaV1.5 channel is positively correlated with cancer stage and identified Na^+ currents (I_{Na}) in different colon tumor-derived primary cells. These findings show that NaV1.5 channels significantly contribute to cancer cell invasiveness; thus, they are promising molecular targets against metastatic disease [66].

It was found that heterologous expression of NaV1.6 channels increases the invasive capacity of cervical cancer cells. In addition, the inhibition of NaV1.6 channel activity by TTX prevented the enhanced invasive capacity of CeCa cells. The invasive capacity of cervical cancer cells associated with NaV1.6 channel activity is mediated by a mechanism in which Na^+/H^+ exchanger-1NHE-1 and the specific secretion and proteolytic activity of MMP-2 matrix metalloproteinase type 2 are involved in this process. Most investigations of metastasis have focused on Nav1.5, particularly in the context of breast cancer [62]. Breast cancer expresses a neonatal splice isoform of Nav1.5 rather than the adult isoform found in normal tissues.

It was shown that the expression of the Nav1.7 channels in Mat-LyLu rat prostate cancer cells but of the neonatal splice form of the Nav1.5 (nNav1.5) channels was strongly correlated with the metastatic condition of the cells. cAMP-dependent protein kinase A (PKA) plays a significant role in the upregulation of the functional expression of these channels, which in turn, this enhances the cell's metastatic potential [70].

Overall, VGSC-related Na^+-current, bioelectrical signals alternate in various types of metastatic cancer cells and play significant roles in regulating cell migration and invasion. Therefore, these channels can be considered key regulators of cancer development and the metastatic cascade and targets in cancer therapy. The results of recent studies are encouraging future research in this direction [71]. Thus, it was demonstrated that the application of the NaV1.7 channel novel inhibitor (SV188) inhibits the *I*Na current and insignificantly withdraws the migration and invasion of aggressive medullary thyroid cancer (MTC) cells at doses lower than its cytotoxic concentration. These data indicate that manipulation of sodium channels can be used to treat metastasis [72].

Potassium (K^+) channels are a large and diverse protein comprising 12 subfamilies that play crucial roles in both excitable and non-excitable cells. The potassium (K^+) channel contains four similar or identical pore-forming α-subunits, each of which contains six transmembrane segments (S1-S6), with the first four voltage sensors containing transmembrane segments (S1-S4). A considerable amount of information obtained from recent studies indicates the important role of K^+ channels in cell proliferation, cancer progression, and migration [21, 73-75]. The potassium channel subfamily proteins, such as K_v, Ether- à-go-go (EAG), and K_{Ca}, $K_{v10.1}$, $K_{v11.1}$, $K_{Ca1.1}$, and $K_{v1.3}$, are the most investigated ion channels. It has been demonstrated that cell hallmark-related properties of the channels are associated with alteration of their expression in primary tumors and metastases cancer [14]. These channels have high expression levels in leukemia, ovarian, lung, and breast cancer cells, among others. The $K_{v10.1}$ channel is overexpressed in more than 70% of tumors and cancer cell lines from the cervix, lung, breast, ovary, neuroblasts, liver, prostate, glial cells, and gastrointestinal tract. Moreover, its crucial role in tumorigenesis, cell signaling, cell cycle regulation, and tumor growth has been demonstrated [76]. There is evidence that $K_{v1.3}$, a voltage-gated K^+ channel, is present in colon and breast cancer, whereas it is absent in normal human tissues [70]. The $K_{v11.1}$ channel participates in the cell cycle and appears as regulators of apoptosis and cell proliferation in cancer cells [77]. It demonstrated high-level expression of the $K_{v11.1}$ channel and its related K^+ current (I_{Kv}) has been observed in different cancer cell lines. The pharmacological and biophysical properties of $K_{v11.1}$ channels (time and voltage dependence of activation and inactivation) were described by characteristic I_{Kr} current. $K_{v11.1}$ channels have only a small input in the hyperpolarization of Vr compared with other K^+ channels, which leads to essentially depolarized Vr in tumor cells compared with normal cells. Related to these findings, it has been shown that the activation of one isoform of the $K_{v11.1}$ HERG1 channel in different cell lines, as well as in primary human tumors, causes more depolarization of cell membrane potential. The study's characteristics of the biophysical properties of K^+ current in cancer cells revealed that it has a small capacity for Vr hyperpolarization. Thus, most likely, there is a K^+ current driven by another mechanism besides membrane depolarization in cancer that provides anti-tumor effects on the channel: 1) regulation of a driving force for Ca^{2+} entry 2) modulation of cell volume, and finally 3) K^+ current-dependent functional interactions within cell signaling molecules [14].

This study investigated the effect of small molecule DCA

(Dichloroacetate) on three human cancer cell lines: A549 (non-small-cell lung cancer), M059K (glioblastoma), and MCF-7 (breast cancer) and compared them with healthy. Results of these studies showed that without affecting normal cells, the application of DCA in cancer cells increased the production of ion current (I_{Kv}). These changes in bioelectrical signals are accompanied by fascinating phenomena. Thus, cancer cells reversed their bioelectrical and apoptotic properties to normal and reduced cell migration. tumor growth. Accordingly, this finding indicates that DCA can act as a manipulator of bioelectrical signals and may be used in the treatment of patients with cancer [16].

Recently, the effects of manipulating K^+ channel-driven bioelectrical signals on triple-negative breast cancer (TNBC) cell invasion and metastasis were investigated using remarkably designed experiments [13]. It was found that by increasing the outward K^+ current, hyperpolarization of RMP in TNBC cells led to increases in breast cancer cell migration and invasion. In the next experiments, the ion current (I_{Kv}) was reduced with the application of the FDA-approved ion channel blocker amiodarone, which led to RPM depolarization. Finally, it was found that amiodarone treatment of TNBC cells decreased the migration rate, number of lung metastases, and the return of cancer cells to a normal state. These studies concluded that cellular RMP has biophysical properties and is very important for pro-migratory signaling pathways in cancer and can be used to identify a new therapeutic target for breast cancer metastasis [13].

BK (Large-conductance Ca^{2+}- and voltage-gated K^+) channels are a unique number of potassium channels that are activated by Ca^{2+}, other signaling molecules (CO, heme, etc.), and/or depolarization of the membrane [78-80]. Accordingly, the channel plays many important physiological roles, including regulation of smooth muscle tone, neurotransmitter release, and neuronal excitability [81-83].

In recent decades, increasing evidence has revealed that BK channels are involved in regulating the cell cycle and proliferation, and they have also been shown to be involved in the migration of cancer cells [84, 85]. Recent studies have reported disorders of BK channels in various types of cancer cells, including triple-negative breast cancer cells, neuroblastoma cells, glioblastoma cells, and human astrocytoma cells [86]. The anti-tumor effects of the BK channel were investigated in breast tumor-bearing MMTV-PyMT transgenic BK knockout mice and a syngeneic transplantation model of breast cancer. These experiments demonstrated the oncogenic actions of BK channels in breast cancer initiation, progression, and tamoxifen sensitivity [86]. Further studies addressing how BK channels promote breast cancer (BC) development and progression using a combination of genetic knockdown BK channels demonstrated that show that BK modulates overall cellular and mitochondrial energy production and mediates metabolic rewiring [87].

It was identified that calcium-dependent large-conductance BK channels are overexpressed in TNBC (triple-negative breast cancer) patients. However, 99% of channels are closed and remain depolarized in TNBC cells. It was demonstrated that opening BK channels hyperpolarizes membrane potential (*Vmem*), induces TNBC cell apoptosis via activated caspase-3, and slows tumor growth. Moreover, these studies revealed that hyperpolarization of *Vmem* by opening the BK channel does not affect healthy breast tissue and cardiac function [88, 89]. These results support the idea that hyperpolarization induced by opening the BK channel in TNBC cells can be a novel strategy for the development of targeted therapies for TNBC [90, 91].

Chloride channels are integral membrane proteins that facilitate the movement of chloride ions accompanying the transport of calcium, sodium, and potassium cations across cellular membranes. Chloride (Cl^-) channels are ion channels activated by voltage, calcium, and pH, providing maintenance of cell membrane potential [14, 24, 92].

Cl^--channels generated currents underlying pathological phenotypes, such as cell proliferation, migration, and metastasis [12]. The biophysical properties of the Cl^- currents (I_{Cl}) during human cell migration were investigated using an in vitro model of invasive migration. It investigated the electrical properties of I_{Cl} when cell volume was alternated and were found to be dependent on the activation of Cl channels. Thus, Inhibition of I_{Cl} with 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB) reduced chemotactic migration in a dose-dependent manner. Time-lapse video microscopy during patch-clamp recordings revealed visible changes in cell shape and/or movement accompanied by spontaneous activation of I_{Cl}. It was suggested that I_{Cl} is activated during cell movement, consistent with the effects of NPPB in migration assays.

Thus, I_{Cl} contributes to the shape and volume changes required for glioma cell migration via brain tissue. These studies revealed that volume-activated I_{Cl} is important for generating the cell size volume changes necessary for the migration of glioma cells [4, 93]. The importance of Cl^--selective channels in migration and invasion was further confirmed in the example of highly metastatic human hepatocellular carcinoma (MHCC97H) cells. It was demonstrated that tamoxifen (antiestrogen anticancer agent) inhibits cell migration and regulatory volume decrease (RVD) in a concentration-dependent manner with a similar *IC50*, which indicates that tamoxifen cells inhibit cell migration by modulating I_{Cl} and volume. Moreover, knockdown of the expression of Cl^--channel proteins using shRNA or siRNA inhibited I_{Cl} and consequently prevented cell migration [94].

In summary, chloride channels are widely expressed and are involved in cell proliferation, apoptosis, and cell cycle. The collected data support the view that the upregulation or downregulation of chloride channel expression in certain types of cancer leads to changes in the biophysical properties associated with the I_{Cl} and membrane potential. There is strong evidence in the literature supporting chloride channels, I_{Cl} plays an important role in cancer cell migration because of

its role in maintaining cell volume. Thus, I_{Cl} is involved in controlling the cell regulatory volume decrease (RVD), which maintains cell viability and promotes invasion and migration. I_{Cl} directly or through Ca^{2+} signaling initiates proliferation signaling pathways that affect cell proliferation and growth [2, 18].

5. Conclusion

While considerable advancement has been made in the understanding of cancer growth and metastasis, the underlying cellular and molecular mechanisms remain poorly understood. Accordingly, efficient therapeutic involvement is still lacking.

Ion channels provide the formation of bioelectrical signals, which are crucial for numerous physiological processes. New studies continue to accumulate evidence supporting the view that paradigm cancer is also characterized by alterations in the function of ion channel-driven bioelectrical signaling circuitry. Recent advancements in the application combination of different biophysical tools provide essential knowledge about these biological functions. Cryo-electron microscopy, crystallography, NMR, EPR spectroscopy, and computer modeling provide atomic-scale snapshots of channel structures in non-cellular environments; however, other biophysical techniques, such as electrophysiology, including voltage-clamp fluorometry, deliver dynamic information that is connected to the functional consequences of bioelectrical signaling mechanisms.

Functionally, disruption of the bioelectrical signaling mechanism triggers cancer development and progression. Consequently, targeting this unique biophysical property of cells has become a fascinating strategy for developing novel anticancer treatments. Recent studies provide a rationale for the future exploration of the role of the molecular-biological modulation of the bioelectrical states of cells in the overriding of powerful oncogenic mutations and the prevention or normalization of tumors.

Abbreviations

A549	Non-small-cell Lung Cancer
AP	Action Potential
M059K	Glioblastoma
RMP	Resting Membrane Potential
RVD	Regulatory Volume Decrease
SDSL	Site-directed Spin Labeling
SDTF	Site-directed Tryptophan Fluorescence
TNBC	Triple-negative Breast Cancer
VGSC	Voltage-dependent Sodium Channels

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Conflicts of Interest

The authors declare no conflicts of interest.

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