Novel insights into ion channels in cancer stem cells (Review)

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Received February 15, 2018; Accepted June 28, 2018

DOI: 10.3892/ijo.2018.4500

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Key words: ion channels, cancer stem cells, tumorigenesis, invasion

Abstract. Cancer stem cells (CSCs) are immortal cells in tumor tissues that have been proposed as the driving force of tumorigenesis and tumor invasion. Previously, ion channels were revealed to contribute to cancer cell proliferation, migration and apoptosis. Recent studies have demonstrated that ion channels are present in various CSCs; however, the functions of ion channels and their mechanisms in CSCs remain unknown. The present review aimed to focus on the roles of ion channels in the regulation of CSC behavior and the CSC-like properties of cancer cells. Evaluation of the relationship between ion channels and CSCs is critically important for understanding malignancy.

Contents

1. Introduction
2. Ion channels and CSCs
3. Ion channel blockers as a novel target for cancer
4. Conclusion

1. Introduction

Cancer stem cells (CSCs). Cancer is a leading cause of mortality worldwide. In China, ~4 million new cases of cancer were diagnosed in 2015, and 50% of all mortalities were associated with cancer (1). Surgery, chemotherapy and/or radiotherapy are used to treat the majority of cancers and to improve survival of patients. These clinical measures have proven efficacious in several cases; however, few patients survive >5 years due to the high recurrence and metastasis of tumor cells; CSCs are considered the root of tumor recurrence and metastasis (2,3).

CSCs have been identified and characterized in various tumor types; in particular, CSCs exhibit self-renewal, multilineage differentiation and tumor initiation capacities, and proliferative potential (4). Targeting of CSCs or inhibition of important properties including self-renewal, differentiation and apoptosis resistance are novel therapeutic strategies (Fig. 1). Several lines of evidence have indicated that CSCs serve a key role in tumorigenesis, recurrence and metastasis (5-7). When tumors occur, CSCs are considered to be the origin of abnormal differentiation; uncontrolled self-renewal of CSCs induces malignant transformation and rapid proliferation of cells. In advanced tumor stages, once activated, CSCs can promote tumor development and metastasis by regulating tumor angiogenesis (8). Notably, the current anti-tumor drugs mainly target rapidly proliferating mitotic cells; however, CSCs are usually dormant or quiescent, and can therefore exhibit immune escape and resist the suppressive effects of chemotherapy drugs, thereby becoming the root of tumor recurrence (3). Therefore, CSCs are considered to be the key to tumor recurrence and metastasis of seed cells and malignant tumors. Previous studies have suggested that there are three major sources of CSCs, as follows: i) Normal stem or progenitor cells are malignantly transformed into CSCs due to gene mutations; ii) viral infection or formation of CSCs through intercellular fusion (9,10); iii) mature end-stage tumor cells regain CSC-like properties induced by ionizing radiation, hypoxia or the tumor microenvironment (11,12). In addition, both inflammatory factors [interleukin (IL) 6, and transforming growth factor (TGF)-β], and cytokines [endothelial growth factor (EGF) and vascular (EGF)] regulate CSC growth and maintenance (Fig. 2).

Ion channels and tumors. Previous studies have reported that ion channels serve an important role in cancer development (13,14). Numerous ion channels have been confirmed to be highly expressed in various tumor types and are closely associated with tumor cell biological behaviors (15-17). Ion channels are specific hydrophilic microporous proteins that exhibit selective permeability for various ions; they are usually named according to the ions with the highest permeability,
including potassium (K⁺) channels, calcium (Ca²⁺) channels and chloride (Cl⁻) channels. These ion channels are distributed in almost every cell membrane of the body and have an important role in the physiology and pathology of excitable cells with regards to the following aspects: i) Determination of cell excitability, conductivity, contractility and rhythmicity; in nerve, muscle and other excitable cells, Na⁺ and Ca²⁺ channels mainly regulate depolarization, whereas K⁺ channels mainly regulate repolarization and maintain the resting potential (18,19); ii) regulation of vasomotor smoothing and contraction potential (20); iii) participation in synaptic transmission (21); iv) maintenance of normal cell volume (22,23); v) regulation of intracellular cAMP, cGMP, Ca²⁺ and other second messenger concentrations, in order to trigger muscle contraction, glandular secretion, protein kinase activation and gene expression regulation (24,25). The normal structure and function of ion channels are the basis for cells to carry out their normal activities. Mutations in specific ion channel sites lead to abnormalities in their activation and inactivation, causing cell dysfunction and the formation of various diseases, including epilepsy and arrhythmia, and skeletal muscle dysfunction (26,27). Disorders associated with aberrant ion channel functions are commonly known as ‘ion channel diseases’ (28,29).

At present, few reports have focused on the association between ion channels and CSCs. Our recent work indicated that solute carrier family 8 member A1 and transient receptor potential cation channel subfamily C member 6 are expressed in cluster of differentiation (CD)133⁺ stem cells in Huh7 hepatic cancer cells, thus indicating that ion channels may be involved in the occurrence and development of cancer (30). Furthermore, ion channel inhibitors can reduce drug resistance of tumor cells via regulation of CSC function (31,32). The present review aimed to summarize the association between ion channels and CSCs. Further evaluation of the association between ion channels and CSCs is critically important to understand malignancy.

2. Ion channels and CSCs

Association between Ca²⁺ channels and CSCs. Cytosolic Ca²⁺ ([Ca²⁺]ₗ) has an important role in intracellular signal transduction, and participates in a series of physiological and pathological processes in the body (33). Under normal circumstances, intracellular Ca²⁺ concentrations are usually maintained within an appropriate concentration range and are regulated by a series of precise regulatory systems (34). However, this balance is disrupted under pathological conditions. It has previously been reported that in the tumor cells, intracellular Ca²⁺ levels may be disrupted, thus affecting the intracellular Ca²⁺ balance, which leads to excessive activation of associated signals that encode alterations in intracellular Ca²⁺ (including source, amplitude and frequency) (35,36). This induces a subsequent upregulation in oncogene expression, which promotes the development of tumors (32). The intracellular Ca²⁺ balance is primarily regulated by Ca²⁺ channels in the membrane and organelles; therefore, it is important to study the role of Ca²⁺ channels and their subtypes in tumorigenesis, development, invasion and metastasis.

There are several known Ca²⁺ channels: Voltage-gated Ca²⁺ channels (VGCCs), ligand-gated Ca²⁺ channels, store-operated Ca²⁺ channels and transient receptor potential channels, and Na⁺/Ca²⁺ channels. VGCCs belong to the CaV family and are involved in Ca²⁺ influx; VGCCs are divided into L-type Ca²⁺ channels, T-type Ca²⁺ channels, P-type Ca²⁺ channels, matrix interaction molecule-1 (STIM1) and the Ca²⁺ release-activated Ca²⁺ channel protein-1 (Orai1). STIM1 and inositol triphosphate receptor combine with membrane uncoupling and Orai to induce Ca²⁺ influx (37,38).

Lee et al revealed that Orai, a key calcium channel for store-operated Ca²⁺ entry, is highly expressed in CSC-enriched populations of human oral/oropharyngeal squamous cell carcinoma (OSCC). In addition, the activation of Orai1 promotes cancer self-renewal via nuclear factor of activated T-cells signaling. Overexpression of Orai1 promotes self-renewal in OSCC and increases the expression of enhancer of zeste homolog 2, Hes1, zinc finger E-box-binding homeobox 2 and interleukin 4. Conversely, inhibition of Orai1 suppresses cancer self-renewal in OSCC (39). Zhao et al detected overexpression of the Ca²⁺ channel α2δ1+ subunit in hepatocellular carcinoma (HCC) CSCs. These HCC CSCs exhibit stem cell-like properties, such as increased self-renewal, increased invasiveness and expression of stem cell-associated genes (octamer-binding transcription factor 4, SRY-box 2, Nanog homeobox and BMI1 proto-oncogene, polycystin ring finger) (40).

Liu et al demonstrated that silencing the expression of transient receptor potential cation channel subfamily M member 7 (TRPM7) in glioma stem cells was able to induce the Notch and signal transducer and activator of transcription 3 pathways, which were downregulated in glioma stem cells. TRPM7 is a ubiquitous ion channel permeable to Ca²⁺ and Mg²⁺. Activation of TRPM7 upregulates the CSC markers aldehyde dehydrogenase 1 (ALDH1) and CD133; TRPM7 activates ALDH1 activity to promote proliferation, migration and invasion of glioma cells (41). In addition, Morelli et al evaluated the abnormal expression of transient receptor potential cation channel subfamily V member 2 (TRPV2) in glioblastoma (GBM) CSCs. TRPV2 was revealed to promote in vitro and in vivo GBM CSC differentiation and inhibit their proliferation (42). Ca²⁺-activated K⁺-channels (BKCa) are associated with a poor cancer prognosis, and are highly expressed in CD133⁺ SH-SY5Y neuroblastoma cells and human GBM CSCs (43). The BKCa channels may be used as a novel marker for GBM, in order to improve the personalization and accuracy of GBM therapy (44). Zhang et al reported that activation of BKCa in rats could promote the transformation of human bone marrow-derived mesenchymal stem cells (MCSs) from G₁ to S phase, and increase the mRNA and protein expression levels of cyclin D1 (45). Conversely, inhibition of BKCa activity was able to downregulate the expression of mRNA and protein expression levels of cyclin D1, thus inhibiting the proliferation of bone marrow MSCs. Inhibition of BKCa with paclitaxel or silencing BKCa reduces cell proliferation in human bone marrow-derived MSCs (45). The Ca²⁺ channels mediating calcium signaling play an important role in proliferation, cell death, migration and invasion during the course of tumorigenesis. Therefore, these findings highlight the elusive role of Ca²⁺ channels and their functions in CSC biology (46).
**K+ channels.** K+ channels are hydrophilic proteins that form microchannels in the cell membrane with specificity for K+. The residue side chains selectively interact with the ions and provide specific permeability barrier function. K+ channels are the most widely distributed and most common class of channels, which participate in the resting potential of action potential repolarization and serve a crucial role in regulating various biological functions in organisms (47-49). These channels include voltage-gated K+ channels (Kv), inward rectifier K+ channel, and BKCa, among others. Kv also includes ether-a-go-go-related gene (ERG), Kv2, Kv7 (KCNQ) K+ channel family, ether-a-go-go family of voltage-gated K+ channels and other common subfamilies (50).

*The role of K+ channels in CSCs.* Kv1.3 (together with KCa3.1) has been implicated in the control of cell proliferation in rat...
MSCs; silencing KCa3.1 inhibits the proliferation of rat bone marrow MSCs by inducing cell cycle arrest at the G0/G1 phase (51). The voltage-sensitive human ERG (hERG, Kv11.1) K+ channel acts as a regulator of proliferation and survival in cancer cells (52,53). The expression of Kv11.1 has been reported in several cancer types, as well as cancer cell lines of different lineages, such as epithelial, leukemia, connective or neuronal cells. Recently, Li et al reported that hERG (Kv11.1) is highly expressed in CD34+/CD38-/CD123 leukemia stem cells (LSCs), interferes with the cell cycle and promotes tumor cell proliferation. Furthermore, the hERG-specific blocker E-4031 inhibits LSC proliferation, by inhibiting G1/S phase transition (54). Another hERG inhibitor, clofilium, destroys the osmotic pressure balance of LSCs intra- and extracellularly via K+-induced cell swelling and rupture. These results suggest that hERG channels may be involved in regulation of the LSC cycle, and that LSCs maintain a constant volume by adjusting osmotic pressure inside and outside of the cell (55).

K+ channel tetramerization domain containing 12 (KCTD12) is a biomarker for clinical prognosis in patients with gastrointestinal cancer following chemotherapy (56). Using a cancer cell-forming test that selects CSCs from the colorectal cancer (CRC) HT29 cell line, Li et al revealed that the expression of KCTD12 is downregulated in the CSC-like cells of CRC. Inhibition of endogenous KCTD12 and overexpression of KCTD12 markedly enhance and suppress CRC cell self-renewal ability, respectively. Furthermore, silencing KCTD12 enhances drug resistance to 5-fluorouracil in HT29 cells (57).

Together, the K+ channel activity is an important event that controls several cellular functions including cell proliferation and cell cycle in CSCs. The results provide evidence for the role of K+ channel and it may be a novel, potential pharmacological target for tumor therapy in the future.

Cl- channels. Cl- channels are the most abundant and physiologically important anion channels in organisms. Their classification is more complex, including voltage-gated (CLC), Ca2+-dependent, swelling-activated Cl- channel, γ-aminobutyric acid-activated (GABA) Cl- channels (58). Various Cl- channels have been reported to exhibit different functions in tumor cells (59). Soroceanu et al detected CLC expression in human malignant glioma cells, which are sensitive to the Cl- toxin chlorotoxin; however, CLC is not expressed in normal tissue. The CLC-specific inhibitor chlorotoxin can inhibit tumor cell invasion of the surrounding tissues, thus suggesting that Cl- channels have an important role in the tumor cell cycle (60).

The role of Cl- channels in CSCs. In recent decades, growing scientific evidence has supported the potential involvement of ion channels in tumorigenesis and carcinogenesis. Setti et al indicated that Cl- intracellular channel protein 1 (CLIC1) is overexpressed in GBM CSCs, where it serves an important role in GBM CSCs self-renewal and proliferation; CLIC1 is primarily detected in the nuclear membrane and in the plasma membrane. In addition, Setti et al demonstrated that...
overexpression of CLIC1 in GBM CSCs is negatively correlated with patient survival. Conversely, silencing CLIC1 inhibits the proliferation, cloning and tumorigenicity of GBM (61). These results may indicate a novel therapeutic approach targeted to GBM. CLIC1 may be considered an attractive target in the CSC population that could finally cure GBM. Compared with CLIC1, CLIC4 is expressed in metastatic CSCs and is associated with the prognostic risks of colorectal cancer (62). In conclusion, Cl- channels may serve an important role in tumor cell migration and tumor metastasis; therefore, Cl- channels may be potential drug targets for the treatment of tumors.

3. Ion channel blockers as a novel target for cancer

In recent years, ion channel drugs have been widely used in clinical practice. It has been reported that various ion channel blockers can affect the proliferation, differentiation, apoptosis and metastasis of tumor cells in numerous types of cancer (58). Inhibiting the K+ efflux can promote apoptosis, and a K+ channel inhibitor may reverse multidrug resistance (MDR) in tumor cells (63). Zhao et al reported that the Ca2+ channel blocker verapamil targets MDR-associated proteins, inhibits pancreatic CSC (gemcitabine-resistant) proliferation and promotes apoptosis of pancreatic cancer cells (64). The specific inhibitor of the Kv1.3 channel aflatoxin (MgTX) and the non-specific inhibitor 4-AP can suppress prostate cancer cell metastasis and lung cancer cell proliferation. Additionally, MgTX can promote prostate cancer cell apoptosis by regulating the transition to the G1-S phase (65). Treatment with the KCa3.1 blocker TRAM-34 and temozolomide (TMZ) is able to significantly reduce DNA synthesis, as well as GBM and CSC survival, compared with TMZ alone. Notably, TMZ/TRAM-34 combination therapy can reduce infiltration of glioma cells (66,67). CSCs isolated from GBM are highly resistant to bis-chloroethylnitrosourea (BCNU) in vitro, whereas the combination of BCNU and a Cl channel inhibitor 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid inhibits the proliferation and promotes apoptosis of BCNU-resistant CSCs (63). CLIC1 is involved in the resistance of BCNU-resistant CSCs and BCNU/DIDS combined-therapy can provide valuable insight for promoting apoptosis or sensitizing glioblastomas to BCNU chemotherapy. These results suggest that CLIC1 may be a drug efflux channel that participates in resistance all approaches of cancer biology, since CSCs have an important role in tumorigenesis, drug resistance (74,75), invasion, metastasis and recurrence. The function of CSCs is predominantly regulated by microenvironmental factors that provide an adaptive landscape for relapsed tumor cells (76-78). Therefore, identifying novel methods for preventing CSC drug resistance could improve the long-term survival of patients. The main factors controlling CSCs include epithelial-mesenchymal transition and the niche environment (79,80). In recent years, the potential regulatory role of ion channels in the tumor microenvironment has been widely recognized, due to the abnormal expression of ion channels in CSCs, and various mechanisms regulating tumorigenesis, malignant transformation and metastasis (81-84). Moreover, those ion channels further induced the aberrant activation of signaling pathways and play important roles in the evolution of cancer development. The PI3K/Akt, JNK, STAT3, Wnt and NF-KB pathways are involved in the self-renewal of CSCs (Fig. 3). These findings have provided novel information, which may aid the eradication of CSCs, improve the efficacy of antitumor drugs and result in a potential cure. Some ion channel agonists or antagonists demonstrate antitumor activity in specific CSCs, which provides a theoretical basis for clinical implementation (83). Additional in-depth research regarding the relationship between ion channels and MDR may lay the foundation for the development of novel agents through drug design and development. Novel perspectives will be gained from the characterization of various ion channel structures and may promote the development of anti-CSC drug targets. It has been hypothesized that through further exploration of the relationship between ion channels and CSCs, ion channels may be revealed to participate in the regulation of CSC pathways, and their inhibitors may provide more information regarding clinical targets in CSC-targeted therapy.

Acknowledgements

The authors would like to thank Professor Biguang Tuo (Department of Gastroenterology, Affiliated Hospital of Zunyi Medical College) for highly professional services.

Funding

The present study was supported by research grants from the National Natural Science Foundation of China (grant no. 81660412 to RX, grant no. 81160265 to JYX, grant no. 81360311 to HJ).

Availability of data and materials

Not applicable.

Authors’ contributions

QC, and AC wrote the manuscript; QD, QL, ZS, CC, XY, YH, JZ, SL, GW, JA and HJ collect the literature; BT and RX. primarily revised and finalized manuscript. JX revised the manuscript for clarity and style.
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