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

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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

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 efficaciousinseveral 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 antitumor 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,

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including potassium (K^+) channels, calcium (Ca^{2+}) 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 Ca^{2+} channels mainly regulate depolarization, whereas K^+ channels mainly regulate repolarization and maintain the resting potential (18,19); ii) regulation of vasomotor smoothing and contraction activities (20); iii) participation in synaptic transmission (21); iv) maintenance of normal cell volume (22,23); v) regulation of intracellular cAMP, cGMP, Ca^{2+} 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 roles of ion channels, and describe their expression and function in CSCs. Further evaluation of the association between ion channels and CSCs is critically important to understand malignancy.

2. Ion channels and CSCs

Association between Ca^{2+} channels and CSCs. Cytosolic Ca^{2+} ($[Ca^{2+}]_{cyt}$) 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 Ca^{2+} 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 Ca^{2+} levels may be disrupted, thus affecting the intracellular Ca^{2+} balance, which leads to excessive activation of associated signals that encode alterations in intracellular Ca^{2+} (including source, amplitude and frequency) (35,36). This induces a subsequent upregulation in oncogene expression, which promotes the development of tumors (32). The intracellular Ca^{2+} balance is primarily regulated by Ca^{2+} channels in the membrane and organelles; therefore, it is important to study the role of Ca^{2+} channels and their subtypes in tumorigenesis, development, invasion and metastasis.

There are several known Ca^{2+} channels: Voltage-gated Ca^{2+} channels (VGCCs), ligand-gated Ca^{2+} channels, store-operated Ca^{2+} channels and transient receptor potential channels, and Na^+/Ca^{2+} channels. VGCCs belong to the CaV family and are involved in Ca^{2+} influx; VGCCs are divided into L-type Ca^{2+} channels, T-type Ca^{2+} channels, P-type Ca^{2+} channels, matrix interaction molecule-1 (STIM1) and the Ca^{2+} release-activated Ca^{2+} channel protein-1 (Orai1). STIM1 and inositol trisphosphate receptor combine with membrane uncoupling and Orai1 to induce Ca^{2+} influx (37,38).

Lee *et al* revealed that Orai, a key calcium channel for store-operated Ca^{2+} 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 Ca^{2+} channel $\alpha 2\delta 1$+ 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, polycomb 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 Ca^{2+} and Mg^{2+}. 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). Ca^{2+}-activated K^+-channels (BK_{Ca}) are associated with a poor cancer prognosis, and are highly expressed in $CD133^+$ SH-SY5Y neuroblastoma cells and human GBM CSCs (43). The BK_{Ca} 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 BK_{Ca} in rats could promote the transformation of human bone marrow-derived mesenchymal stem cells (MCSs) from G_1 to S phase, and increase the mRNA and protein expression levels of cyclin D1 (45). Conversely, inhibition of BK_{Ca} 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 BK_{Ca} with paxilline or silencing BK_{Ca} reduces cell proliferation in human bone marrow-derived MSCs (45). The Ca^{2+} 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 Ca^{2+} channels and their functions in CSC biology (46).

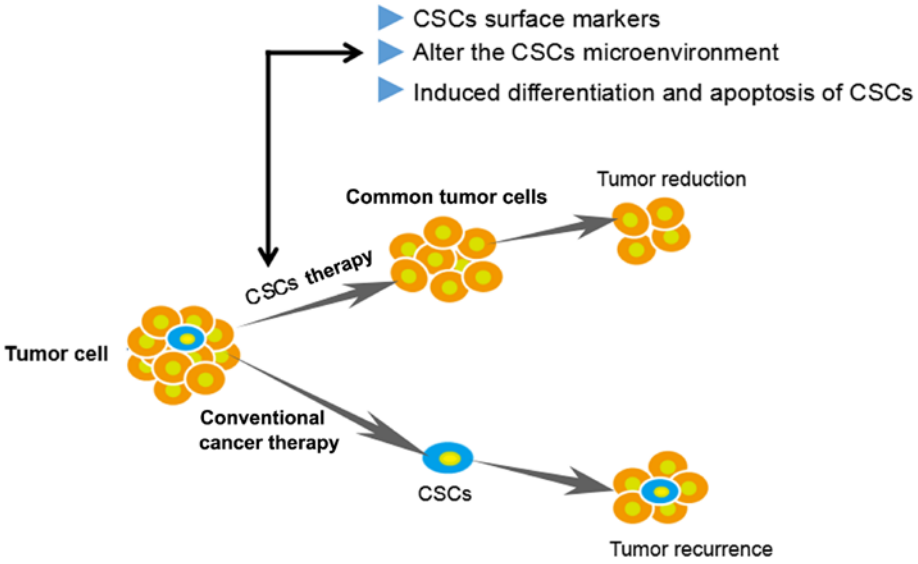

Figure 1. Evolution of CSCs and conventional cancer therapies. Malignant tumors are commonly seen in many human organs, and several traditional methods are used to treat these cancers, including surgery, chemotherapy and radiation. The CSC is an ideal target cell, which possesses self-renewal and multi-lineage differentiation capabilities. CSCs can escape the suppressive effects of normal drugs, and promote tumor recurrence and metastasis. CSC-targeted treatment aims to target specific surface markers of CSCs, affect the CSC microenvironment and induce differentiation and apoptosis of CSCs. These methods may reduce tumor recurrence, which is the root cause of invasion, consequently shrinking the tumor. CSCs, cancer stem cells.

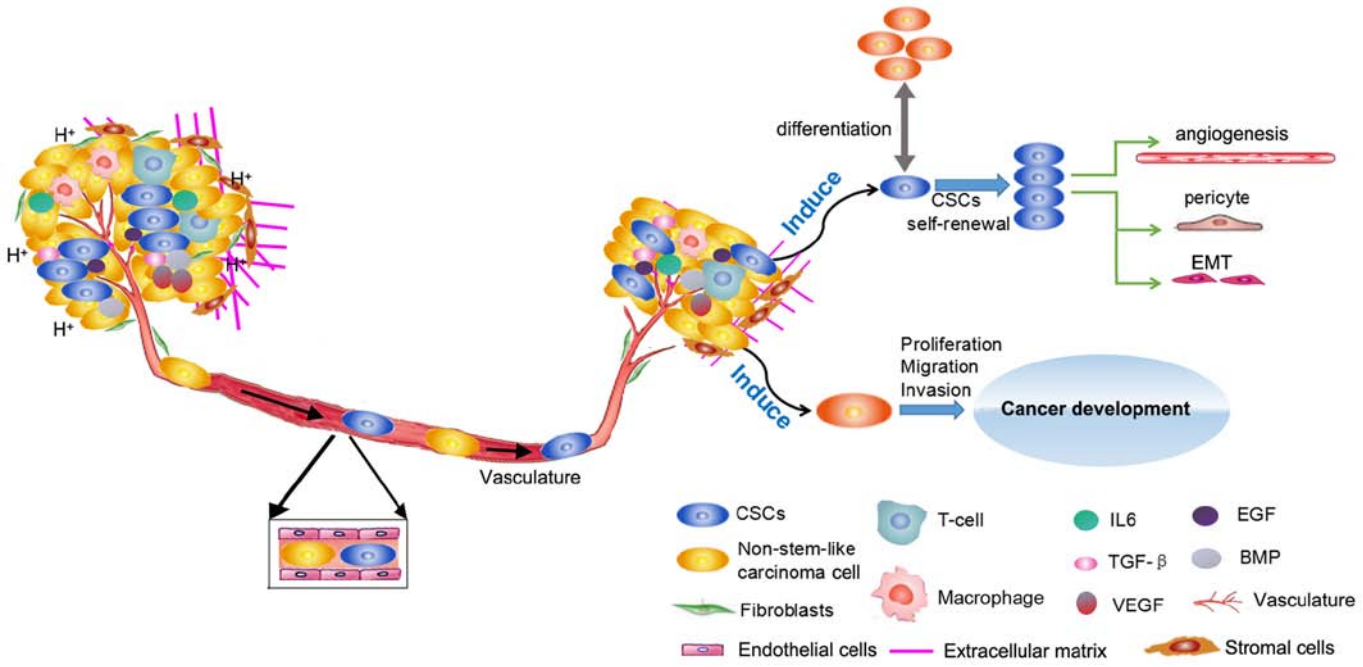

Figure 2. Microenvironment contributes to tumor progression and metastasis. The tumor microenvironment includes tumor cells, CSCs, stromal cells, fibroblasts, tumor vasculature and extracellular matrix, etc. These cells can secrete IL6, BMP, EGF, VEGF, TGF-β and other factors [e.g. neuroendocrine (NE) cells, adipose cells, and the blood networks]. These factors induce the self-renewal and pluripotent differentiation capabilities of CSCs. Furthermore, these factors promote tumor development via stimulating angiogenesis and EMT. BMP, bone morphogenetic protein; CSCs, cancer stem cells; EGF, epidermal growth factor; EMT, epithelial-mesenchymal transition; IL6, interleukin 6; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor.

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 BK_{Ca}, among others. Kv also includes ether-a-go-go-related gene (ERG), Kv2, Kv7 (KCNQ) K^+ channel family, *ether-à-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

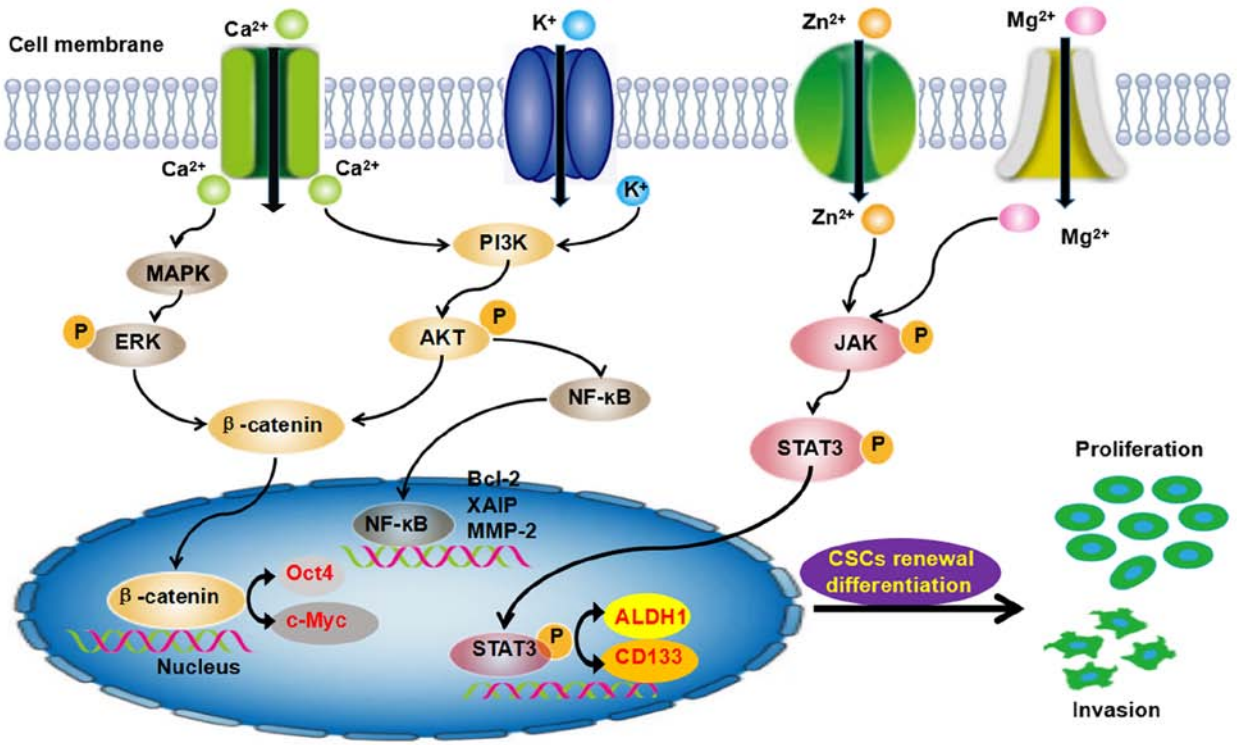

Figure 3. Overview of select signaling pathways associated with CSC maintenance, survival, proliferation and invasion. Ion channel expression in the CSCs membrane, including Ca^{2+}, Mg^{2+}, K^+ and Zn^{2+} channels. These channels are activated and transmit signals through other factors, such as MAPK/ERK, PI3K/Akt, JNK, STAT3, Wnt and NF-κB. These novel regulatory mechanisms may promote self-renewal and differentiation, and thereby provide avenues for therapeutic intervention. MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; NF-κB, nuclear factor-κB; ALDH1, aldehyde dehydrogenase 1; Bcl-2, B-cell lymphoma 2; CD133, cluster of differentiation 133; CSCs, cancer stem cells; JAK, Janus kinase; MMP-2, matrix metalloproteinase 2; Oct4, octamer-binding transcription factor 4; STAT3 , signal transducer and activator of transcription 3; XIAP, X-linked inhibitor of apoptosis protein.

MSCs; silencing KCa3.1 inhibits the proliferation of rat bone marrow MSCs by inducing cell cycle arrest at the G_0/G_1 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, leukemic, 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 G_1/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-fluoruracil 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), Ca^{2+}-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 Ca^{2+} 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 G_1-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 the resistance of GBM CSCs to BCNU (68). In addition, the use of a blocker [5-nitro-1-(3-phenylpropyl amino) benzoic acid] or small interfering RNA silencing of CLCN3 Cl^- volume sensitive channel expression, as well as mRNA and protein downregulation of cyclin D and E, inhibits MSCs proliferation *in vitro*. Furthermore, Gritti *et al* revealed that metformin can inhibit CLIC1 channel function and reduce the survival of human GBM CSCs, and short hairpin RNA against CLIC1 significantly increases the inhibitory effects of metformin on human GBM CSC activity (69). In addition to K^+ and Cl^- channel inhibitors, Ca^{2+} channel inhibitors may reverse cancer cell MDR (70,71).

4. Conclusion

The novel concept of CSCs was introduced in the late 1990s, and numerous research efforts have aimed to elucidate its role over the past decades (72,73). This concept may influence 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.

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Availability of data and materials

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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.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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