

Targeting potassium channels in cancer

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Potassium channels are pore-forming transmembrane proteins that regulate a multitude of biological processes by controlling potassium flow across cell membranes. Aberrant potassium channel functions contribute to diseases such as epilepsy, cardiac arrhythmia, and neuromuscular symptoms collectively known as channelopathies. Increasing evidence suggests that cancer constitutes another category of channelopathies associated with dysregulated channel expression. Indeed, potassium channel–modulating agents have demonstrated antitumor efficacy. Potassium channels regulate cancer cell behaviors such as proliferation and migration through both canonical ion permeation–dependent and noncanonical ion permeation–independent functions. Given their cell surface localization and well-known pharmacology, pharmacological strategies to target potassium channel could prove to be promising cancer therapeutics.

Introduction

Cancer represents a major public health problem and is a leading cause of death worldwide. As reported by the American Cancer Society, more than 1.6 million new cases were diagnosed in 2013, and one in four deaths in the US is cancer related. The standard of care for many cancers typically includes surgery, chemotherapy, and radiation therapy. Although these traditional clinical measures have proven their efficacy in cancer treatment, patients often experience debilitating side effects that significantly reduce their quality of life. In addition, cancer relapse with treatment resistance underscores the urgent need to identify novel molecular targets for the development of alternative therapies.

Ion channels are transmembrane proteins that regulate the flow of ions across biological membranes. About 13% of currently known drugs whose primary therapeutic targets are ion channels are being used for the treatment of a variety of human conditions, including cardiovascular and neurological disorders (Overington et al., 2006). Although our knowledge of ion channel functions in the physiological and pathological conditions

of excitable cells such as cardiomyocytes and neurons is vast, it is only recently that the manifold functions of ion channels in driving malignant cancer cell behaviors have been reported. Potassium channels represent the most diverse group of ion channels, and have enormous potential as therapeutic targets for personalized cancer treatment, given their huge functional and structural diversity. In this review we summarize the dysregulated potassium channel expression in cancer, discuss the complex mechanisms potassium channels use for regulating cancer cell proliferation and migration, illustrate the therapeutic value for targeting potassium channel in preclinical models, and outline the major unanswered questions in the field. We direct the readers to excellent reviews on the functional roles of other ion channel classes in cancer (sodium channels: Roger et al., 2006; Djamgoz and Onkal, 2013; Fraser et al., 2014; calcium channels: Yang et al., 2010; Monteith et al., 2012; TRP channels: Prevarskaya et al., 2011; Santoni and Farfariello, 2011; Ouadid-Ahidouch et al., 2013; chloride channels: Cuddapah and Sontheimer, 2011).

Potassium channels

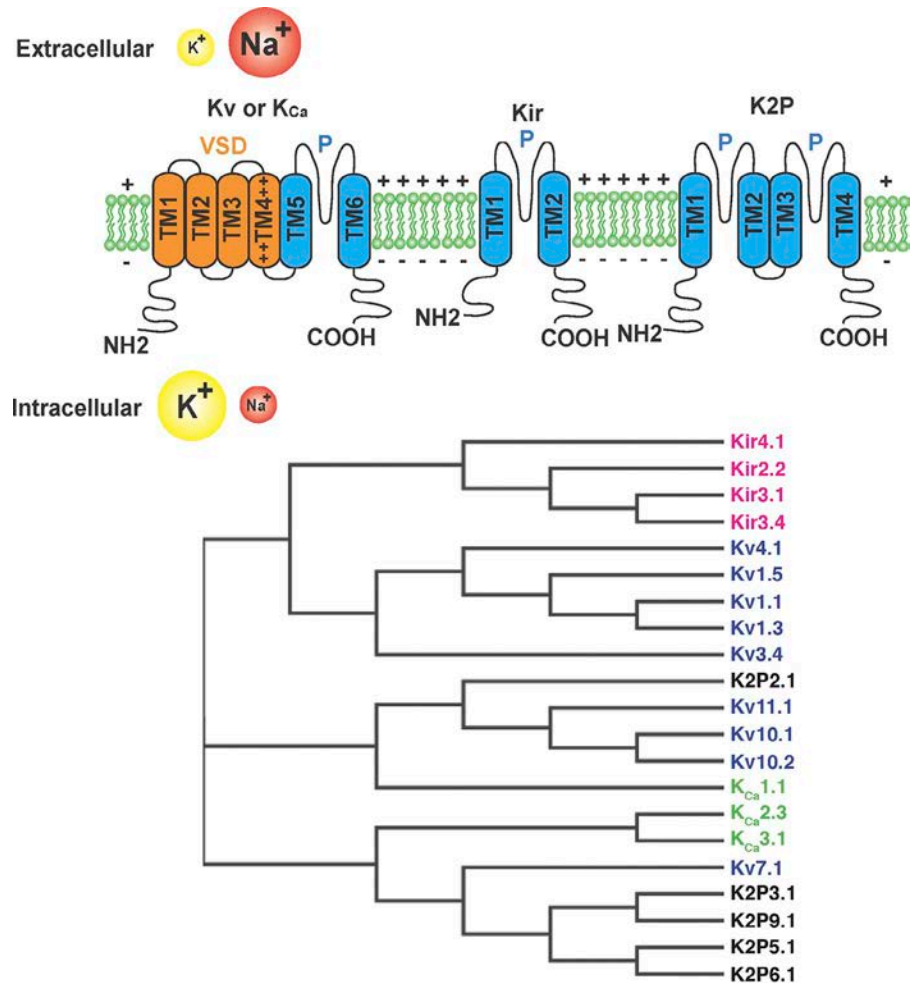
The >400 ion channel–encoding genes represent ~1.5% of the human genome, and ion channels display vast structural and functional diversity. These channels are central regulators of the distribution of potassium, sodium, calcium, and chloride ions for cellular ionic homeostasis and contribute to essentially all fundamental cellular processes. Here we focus on potassium channels, which selectively conduct potassium ions across the membrane down their electrochemical gradient. Because the sodium–potassium pump ($\text{Na}^+\text{-K}^+\text{-ATPase}$) transports two potassium molecules into the cell and three sodium molecules out of the cell at the cost of hydrolyzing one ATP molecule, this results in a higher intracellular potassium concentration. Once a potassium channel opens, it allows potassium ions to flow down its concentration gradient. This potassium conductance will drive the cell membrane potential toward the equilibrium potential for potassium. In nonexcitable cells or excitable cells at their basal state, the resting membrane potential is generally maintained at negative values ranging from -30 to -85 mV. The membrane potential can either become more negative (a process called hyperpolarization) or more positive (a process called

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Figure 1. Potassium channels involved in oncogenic processes belong to all four classes.

(Top) Calcium-activated potassium channels (K_{Ca}) and voltage-gated potassium (Kv) channels are composed of four pore-lining α subunits. Whereas SK and IK channels resemble Kv channels in having six transmembrane segments (TM1–TM6) and a reentrant pore loop between TM5 and TM6 per α subunit, BK channels have one additional transmembrane segment in the N terminus of the α subunit whereas its cytoplasmic C-terminal domain confers the channel's calcium sensitivity. The first four transmembrane segments (TM1–TM4) of Kv channel α subunit form the voltage sensor domain, whereas the remainder of the transmembrane domain corresponds to the pore domain conserved in all potassium channels (Isacoff et al., 2013). The inward rectifying potassium channel (Kir) possesses two transmembrane domains with a pore-forming region (P) in between. The K2P channel is the so-called “background” potassium channel that is made of four transmembrane domains and two pore-forming regions. All potassium channels require a tetrameric arrangement of the pore-forming regions of the α subunits to form the potassium-selective filter, therefore a complete conductive channel is a tetramer for the one-pore channels (Kv, K_{Ca} , and Kir) or dimer for the K2P channels. The resting cell membrane potential is hyperpolarized due to the imbalance of potassium and sodium ion distribution caused by the electrogenic function of Na^+K^+ -ATPase. (Bottom) Phylogenetic dendrogram shows that the various potassium channels involved in oncogenic processes belong to all four classes.



depolarization) compared with the resting potential as various ion channels open. Because potassium channels dominate the ion conductance at the resting state, the resting membrane potential is slightly more positive than the equilibrium potential for potassium. So, in general, potassium channel activation would cause hyperpolarization, whereas opening of sodium channels or chloride channels would cause depolarization.

With 78 members, potassium channels can be divided into four main classes based on their domain structure and activation mechanisms (Fig. 1). Voltage-gated potassium channels (Kv), encoded by 40 genes in humans, are the largest subset of potassium channels gated by changes in the membrane potential. Calcium-activated potassium channels (K_{Ca}) are activated by intracellular calcium. According to their conductance, K_{Ca} channels can be further divided into big conductance (BK), intermediate conductance (IK), and small conductance (SK) channels. Kv and K_{Ca} channels share a similar domain organization and are composed of four pore-lining α subunits, each with six transmembrane domains (TMs) and one pore-forming region, except for the BK channel, which has one additional transmembrane segment in the N terminus. The TM1–4 represents the voltage-sensing domains (VSDs) for Kv channels (Fig. 1). Inward rectifying potassium channels (Kir) possess two transmembrane segments flanking one pore loop in each of the four α subunits (Fig. 1), and they pass potassium ions more

easily in the inward direction (into the cell) than the outward direction (out of the cell). Two-pore domain potassium channels (K2P) have two pore domains per α subunit, each pore domain with two transmembrane segments flanking a reentrant pore loop (Fig. 1). Two such α subunits form a K2P channel that is usually constitutively open as a “leak channel” for maintaining a negative membrane potential. While the pore domain of all potassium channels possesses a highly conserved GYG/GFG signature motif, the molecular diversity of potassium channels is immense. Potassium channels form homomeric or heteromeric complexes assembled with α subunits from the same subfamily. The presence of auxiliary subunits can modify the functional properties and further diversify these channels. For example, a BK channel can be activated at resting membrane potential without an increase of intracellular calcium concentration by forming a complex with an auxiliary protein (Yan and Aldrich, 2010). Potassium channel mRNAs are subjected to RNA editing (Bhalla et al., 2004; Garrett and Rosenthal, 2012; Ryan et al., 2012) and alternative splicing (Schwarz et al., 1988; Xie and McCobb, 1998; Sun et al., 2009), and the channel proteins can be further modified at the posttranslational level by phosphorylation (Park et al., 2008), sumoylation (Plant et al., 2010) (Plant et al., 2011), palmitoylation (Shipston, 2011), and glycosylation (Khanna et al., 2001; Gong et al., 2002). These RNA and protein modifications together with the

Table 1. Potassium channel expression in cancer

Cancer type	Channel gene																					
	Kv1.1 KCNA1	Kv1.3 KCNA3	Kv1.5 KCNA5	Kv3.4 KCNC4	Kv4.1 KCND1	Kv7.1 KCNQ1	Kv10.1 KCNH1	Kv10.2 KCNH5	Kv11.1 KCNH2	Kca1.1 KCNMA1	Kca2.3 SK3	Kca3.1 IK1	Kir2.2 KCNJ12	Kir3.1 KCNJ3	Kir3.4 KCNJ5	Kir4.1 KCNJ10	Kir6.1 KCNJ8	Kir6.2 KCNJ11	K2P2.1 KCNK2	K2P3.1 KCNK3	K2P5.1 KCNK5	K2P9.1 KCNK9
Adrenal																						
Blood																						
Bone																						
Brain																						
Breast																						
Cervix																						
GI tract																						
Head and neck																						
Kidney																						
Lung																						
Lymphoma																						
Melanoma																						
Ovary																						
Pancreas																						
Prostate																						
Sarcoma																						
Thyroid																						
Uterine																						

Expression of various potassium channels that have been implicated in cancer. Red boxes indicate that overexpression is reported (in most cases) and that the channel enhances tumorigenic processes, for example by driving proliferation, cell migration, or metastasis (Bielanska et al., 2009; Williams et al., 2013; Pardo and Stühmer, 2014). Green boxes indicate that the channel expression is inversely correlated with tumor malignancy and clinical aggressiveness, such as KCNA5 in glioma (Preussat et al., 2003) and lymphoma (Bielanska et al., 2009), and KCNQ1 in colorectal cancer (Than et al., 2013). The blue box indicates the presence of recurring somatic mutations in the KCNJ5 gene detected in adrenal adenomas (Choi et al., 2011). GI, gastrointestinal.

mix and match of channel subunits afford tremendous versatility of channel behaviors.

Dysregulated potassium channel expression in cancer

Numerous studies have reported dysregulated potassium channel expression in human cancer. These potassium channels implicated in oncological processes belong to all four main classes (Fig. 1). For example, overexpression of the voltage-gated potassium channel Kv1.1 marks a subgroup of medulloblastoma (Taylor et al., 2012); elevated Kv1.3 expression is detected in a number of human malignancies including breast, colon, and prostate cancer (Comes et al., 2013); high Kv11.1 (HERG) expression marks both solid and blood cancer (Pillozzi et al., 2002; Jehle et al., 2011); and Kv10.1 (EAG1) overexpression is found in >70% human cancer types of various organs (Hemmerlein et al., 2006). Overexpression of the G-protein-activated inwardly rectifying potassium channel GIRK1 (KCNJ3) is correlated with the presence and degree of breast cancer lymph node metastases (Stringer et al., 2001). Overexpression of a specific splice isoform of the BK channel correlates with the malignancy grade of glioma (Liu et al., 2002). The K2P channel KCNK9 is overexpressed in breast and lung cancer (Mu et al., 2003). A detailed summary of potassium channel expression is shown in Table 1.

Does the dysregulated potassium channel expression (mostly overexpression) initiate tumorigenesis, confer advantages to malignant growth or metastatic spread once the tumor forms, or is the aberrant expression simply a consequence of neoplastic transformation without functional significance? Two potassium channels have been shown to possess tumorigenic

capacity when ectopically expressed in heterologous systems. Aggressive tumor growth is induced by transfecting EAG1 into Chinese hamster ovary (CHO) cells (Pardo et al., 1999), or retroviral induction of KCNK9 expression in normal mouse mammary gland epithelial cells and mouse embryonic fibroblast cells (Mu et al., 2003). However, these cell lines already possess unlimited proliferative potential in culture. So it is important to determine whether overexpression of a specific potassium channel in situ of an animal model is tumorigenic, which is a more stringent criterion for an oncogene. Considering the fact that pharmacological inhibition or genetic suppression of potassium channels reduces growth in multiple cancer types (Pardo and Stühmer, 2014; Urrego et al., 2014), it is possible that most potassium channels take up a “permissive” role to mostly function as essential regulators for various cancer cell behaviors such as proliferation and migration, and that their up-regulation provides a repertoire for sufficient channel activity when necessary. Strikingly, up-regulation of the plant orthologue of Shaker-like voltage-gated potassium channel AKT1 has been detected in *Arabidopsis thaliana* tumors induced by bacterial infection, and the plant tumor growth is reduced in AKT1 channel mutants (Deeken et al., 2003), highlighting the evolutionarily conserved mechanism for potassium channels to drive tumor growth. Of note, although the expression of many potassium channels is up-regulated upon neoplastic transformation compared with adjacent normal tissue, low expression of the voltage-gated potassium channel KCNQ1 is significantly associated with poor survival in patients with colorectal cancer (Than et al., 2013). Consistently, loss of Kcnq1 in a mouse model of intestinal tumor resulted in more aggressive cancer (Than et al., 2013), which suggests that KCNQ1 may function as a tumor suppressor.

This notion is further supported by the finding that misexpression of KCNE1, the regulatory accessory subunit that inhibits KCNQ1 activity, leads to hyperproliferative and invasive phenotypes in *Xenopus laevis* embryonic stem cells (Morokuma et al., 2008). Recent work has demonstrated that oncogenic stress increases KCNA1 expression and promotes its relocation from the cytoplasm to the plasma membrane, which is required for oncogene-induced senescence. Ectopic expression of KCNA1 inhibits RAS-induced transformation, whereas a reduction of KCNA1 expression correlates with an increase in breast cancer aggressiveness (Lallet-Daher et al., 2013). These findings suggest that KCNA1 may restrict tumor growth through a potassium channel-dependent senescence pathway, and the functional significance of potassium channel during tumorigenesis could vary with the channel and/or cancer type.

Dysregulation of potassium channel expression has been identified at genomic, transcriptional, posttranslational, and epigenetic levels. For example, KCNK9 is amplified from 3- to 10-fold in 10% of breast tumors (Mu et al., 2003), and genomic amplification of EAG1 was found in 15% of head and neck carcinoma (Menéndez et al., 2012) and 3.4% of human colorectal adenocarcinoma (Ousingsawat et al., 2007). Estrogen treatment can up-regulate Kir channel transcription in human breast cancer cells to promote proliferation (Williams et al., 2008). An epigenome-wide study has shown that epigenetic silencing of HERG expression by hypermethylation is a good prognostic marker in ovarian clear cell carcinoma (Cicek et al., 2013), which is consistent with the notion that this channel promotes malignant tumor growth. Interestingly, recent cross-cancer analysis of DNA methylation patterns has revealed that voltage-gated potassium channels are frequently hypermethylated. For example, KCNA3 hypermethylation is prominent in breast, lung, colorectal, kidney, ovarian, and prostate cancer (Brevet et al., 2009a,b; Kim et al., 2012). The correlation between KCNA3 hypermethylation to its expression and the clinical significance of this epigenetic alternation remains to be determined. As described above, KCNK9 amplification is detected in human carcinomas, and its ectopic expression confers tumorigenicity to otherwise nonneoplastic cell types (Mu et al., 2003). However, its expression correlates with prolonged survival after ovarian carcinoma resection (Innamaa et al., 2013). In addition, although HERG channel blockers inhibit the growth of multiple cancer cell types, application of the HERG channel activator also impairs mammary gland adenocarcinoma growth by inducing a senescence program (Lansu and Gentile, 2013). It is therefore important to note that the biological significance of potassium channel expression in cancer and whether its activation is tumorigenic or tumor suppressive is context dependent.

Potassium channel in proliferation and tumor growth

Historically, the involvement of potassium channels in cell proliferation was reported by the seminal work of DeCoursey et al. (1984), who demonstrated that voltage-gated potassium channels are the predominant channels in proliferative human T lymphocytes. Application of mitogenic factor promoted channel opening at a more negative membrane potential, and application

of potassium channel blockers inhibited DNA synthesis (DeCoursey et al., 1984). This discovery prompted investigations of potassium channel physiology during cell cycle progression. Indeed, early studies of unfertilized mouse oocytes have shown that a large-conductance potassium channel is active during the M and G1 phases and becomes inactivated during S and G2 phases. This temporal modulation of channel activity is not dependent on cell cycle-specific protein synthesis. Interestingly, the regulated potassium channel activity is correlated with a membrane potential oscillation, in which the cell is more depolarized at S and G2 (Day et al., 1993). Since then the cell cycle phase-dependent potassium channel expression, localization, and activity have been found in multiple cell types (Takahashi et al., 1993; Arcangeli et al., 1995; Brüggemann et al., 1997; Ouadid-Ahidouch et al., 2004; Huang et al., 2012), which suggests that it may be a general phenomenon that accompanies proliferation. How does a potassium channel regulate cell cycle progression? Emerging evidence has led to the proposal of four main mechanisms: setting up membrane potential oscillation, controlling the cell volume dynamics, regulating calcium signaling, and promoting malignant growth via noncanonical function independent of ion permeation (Fig. 2).

Compared with the more hyperpolarized resting membrane potential in differentiated cell types such as neurons or cardiomyocytes (-60 to -80 mV), rapidly proliferating embryonic cells, stem cells, or cancer cell are in general more depolarized, with a resting membrane potential at -20 to -40 mV (Yang and Brackenbury, 2013). Therefore, one classical hypothesis is that membrane depolarization itself plays a functional role in tumor initiation or progression (Fig. 2). In support of this hypothesis, sustained depolarization induces new DNA synthesis, promotes mitotic activity in terminally differentiated central nervous system neurons cultured in vitro (Cone and Cone, 1976), and prevents mesenchymal stem cell differentiation (Sundelacruz et al., 2008). Depolarized membrane potential also induces tumor-like growth and invasion in *Xenopus* embryos (Morokuma et al., 2008). As potassium conductance is the predominant regulator for setting up the resting membrane potential, the potassium channel composition in cancer cells should accommodate the necessity of maintaining a relatively depolarized state. Because the identities of potassium channels vary among different cancer types, the mechanism underlying the different potassium channels' homeostatic control to achieve universal membrane depolarization remains to be elucidated. Although the membrane potential of cancer cells tends to be more depolarized, cell cycle phase-specific changes in membrane potential have been reported. MCF-7 human mammary cells display a transient hyperpolarization at G1-S transition, which was suggested to be a result of an increase in membrane permeability to potassium (Wonderlin et al., 1995; Fig. 2). Neuroblastoma cells exhibit hyperpolarization at G1-S transition and depolarization at G2-M transition, and the hyperpolarization phase correlates with increased potassium efflux (Boonstra et al., 1981; Fig. 2). Transition from quiescence to mitosis in mouse lymphocytes is accompanied by an initial phase of depolarization, followed by hyperpolarization during DNA replication (Kiefer et al., 1980; Fig. 2). Because mouse oocytes display membrane depolarization

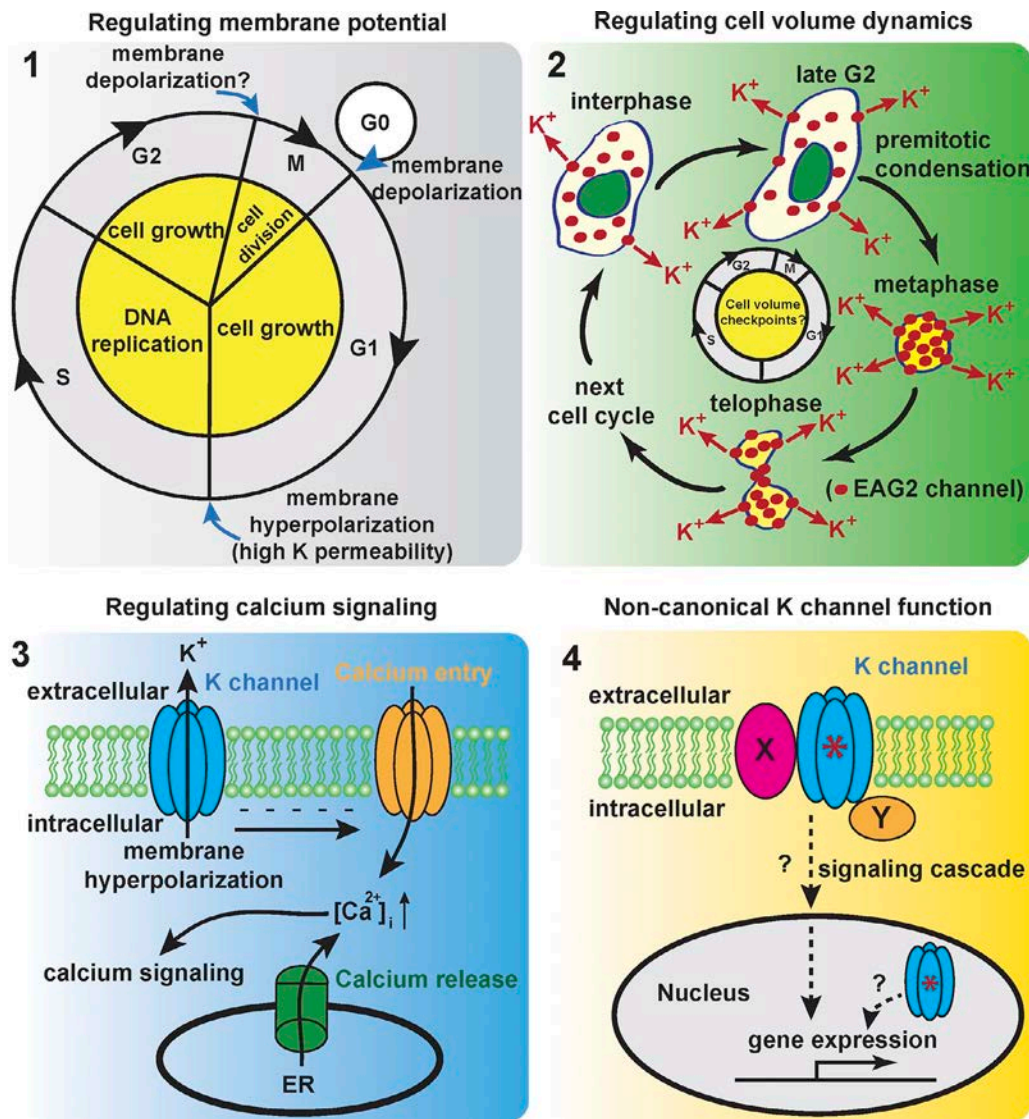


Figure 2. Proposed mechanisms for potassium channels to regulate cell proliferation. Potassium channels may regulate cell proliferation by four mechanisms: setting up oscillating membrane potential, controlling cell volume dynamics, regulating calcium signaling, and promoting malignant growth via noncanonical function independent of ion permeation. (1) Membrane potential oscillation has been reported in multiple proliferative cell types. A transient hyperpolarization at G1–S transition is accompanied by increased potassium channel activity. Depolarization can induce mitotic activity in several types of terminally differentiated cells. A transient depolarization at G2–M transition has been observed. Whether the potassium channel is required and the physiological significance of this depolarization at G2–M transition remain unclear. (2) Modified from Huang et al. (2012): Potassium channels can regulate cell proliferation via controlling cell volume dynamics throughout the cell cycle. This is exemplified by the voltage-gated potassium channel EAG2 that exhibits cell cycle phase-specific membrane localization. EAG2 localizes intracellularly during interphase but enriches at the plasma membrane during late G2 and mitosis. This temporal EAG2 membrane association promotes potassium efflux for premitotic condensation that is essential for mitotic entry, as well as regulating mitotic morphology for successful cell cycle progression. Temporally and spatially regulated potassium channel functions therefore may be critical for passing the “cell volume checkpoint” for successful cell cycle progression. (3) Potassium efflux through potassium channel can lead to membrane hyperpolarization, which increases the driving force for calcium entry through a calcium-permeable channel at the plasma membrane. This calcium entry can trigger calcium release from the internal store. Increased intracellular calcium concentration ($[Ca^{2+}]_i$) can elicit calcium signaling to promote cell proliferation. (4) The potassium channel can regulate cell proliferation through a noncanonical function independent of its potassium ion permeability (indicated by the channel with red asterisks). The potassium channel can interact with membrane protein (X) or intracellular protein (Y) to initiate signaling cascade. Alternatively spliced potassium channel lacking channel domains can enter the nucleus to modulate cell function (Sun et al., 2009). How potassium channels exert noncanonical function to regulate cell proliferation remains largely unknown.

at S and G2 phase (Day et al., 1993), the dynamic changes of membrane potential during cell cycle progression likely are cell type dependent. It is important to consider that, although cancer cells are in general more depolarized, a significant deviation from the range of their physiological membrane potential should also be detrimental. Consistent with this notion, robust membrane depolarization mediated by optogenetic stimulation

in ChETA (engineered channelrhodopsin-2 variant)-expressing glioma cells significantly reduced intracranial tumor growth and enhanced mouse survival (Yang et al., 2013). Induction of the expression and activity of a constitutively open mutant sodium channel also leads to rapid and robust cell killing in multiple tumor cell types (Lemaire and Halperin, 2009), with the mechanisms potentially involving both membrane depolarization

and osmotic shock. Conversely, persistent hyperpolarization in glioma cells induced with Kir4.1 channel expression results in significant growth arrest, a phenotype that can be inhibited by the application of the Kir blocker barium (Higashimori and Sontheimer, 2007).

Besides the direct impact of membrane potential changes on tumorigenesis, potassium channel activity can hyperpolarize the membrane to increase the driving force for calcium entry through calcium-permeating channels (Fig. 2). For example, PDGF up-regulates IK channel expression in vascular smooth muscle cells, and IK regulates PDGF-induced proliferation by promoting calcium entry and calcium-dependent signaling (Bi et al., 2013). Interestingly, chronic pharmacological activation of IK channels paradoxically inhibits PDGF-induced proliferation by suppressing the rise of intracellular calcium concentration and its own expression (Bi et al., 2013), highlighting the importance of temporal and spatial regulation of potassium channel activity and calcium entry for successful cell proliferation. Calcium is a major second messenger that can modulate a multitude of fundamental cellular functions such as proliferation, migration, survival, and apoptosis that have an impact on various aspects of tumor progression. Recent excellent reviews have discussed the functional involvement of calcium signaling in cancer biology (Monteith et al., 2007, 2012). Many potassium channels demonstrate cell cycle-dependent expression level, trafficking, or activity. Studying potassium channel function together with the advanced membrane potential monitoring and calcium imaging techniques throughout cell cycle progression in specific cell types should provide greater understanding of this complex and dynamic process.

Mammalian cells undergo marked cell volume and shape changes during cell cycle progression. Comprehensive studies of volume dynamics have shown that the cells increase volume at interphase, rapidly condense cytoplasmic volume before mitotic entry (premitotic cytoplasmic condensation; PMC), and reach a minimal volume at metaphase to achieve mitotic rounding, followed by volume increase and cytokinesis to generate two daughter cells (Habela and Sontheimer, 2007; Boucrot and Kirchhausen, 2008; Fig. 2). By governing potassium ion flow and intracellular osmolarity that drive obligatory water flow across cell membrane, potassium channels are central regulators for cell volume. Using a genome-wide microarray study, we have found that Kv10.2 (EAG2) overexpression is a hallmark of a subset of medulloblastomas across different molecular and histological subgroups. The EAG2 channel displays dynamic distribution throughout the cell cycle and enriches at the plasma membrane at G2 and M phase. This selective membrane association of EAG2 is accompanied by elevated outward potassium current (Fig. 2). Consistent with the hypothesis that the outward potassium flow drives cell volume reduction for PMC and mitotic entry, genetic suppression of EAG2 expression results in significantly increased cell volume at late G2 phase and induces G2 arrest. For the few medulloblastoma cells with deficient EAG2 function that escape the G2 arrest and enter mitosis, they fail to achieve proper mitotic morphology and encounter mitotic catastrophe. The medulloblastoma tumors with deficient EAG2 function display markedly impaired

in vivo growth. Conversely, overexpressing EAG2 in heterologous systems (HEK293 and COS7 cells) results in constitutive membrane localization of EAG2 and sustained cell volume reduction, which also impairs cell cycle progression and leads to apoptosis (Huang et al., 2012). Taken together, these results demonstrate that EAG2 regulates cell volume dynamics important for cell cycle progression. Cell volume regulation involves concurrent flux of potassium and chloride ions to accompany water flow. While the identify of anion-conducting channels involved in medulloblastoma cell volume regulation remains to be determined, the CLC3 chloride channel has been shown to conduct outward chloride current to drive regulatory volume reduction in glioma cells, and inhibiting CLC3 channels reduces proliferation (Habela et al., 2008). Because cell volume oscillation appears to be a universal phenomenon during cell cycle progression, identifying and targeting specific ion channels regulating this process offers great therapeutic potential.

Potassium channels form macromolecules at the plasma membrane. Besides the membrane-spanning potassium ion permeation pathway, potassium channels could employ their cytoplasmic domains for protein–protein interaction. It is therefore conceivable that some functional roles the potassium channels play during oncological process are mediated by these intracellular elements independent of their capacity to pass potassium ions (Fig. 2). Consistent with this notion, transfecting wild-type and nonconducting *Drosophila melanogaster* eag channels into NIH3T3 cells induced comparable levels of proliferation (Hegle et al., 2006). Furthermore, expressing a nonconducting mutant EAG1 (Downie et al., 2008) or Kv1.3 (Cidad et al., 2012) is sufficient in promoting allografted tumor growth. However, the ability for KCNK9 (Pei et al., 2003) to regulate cancer cell behaviors depends on its canonical role in potassium conductance, which suggests that there are multiple underlying mechanisms for the potassium channel to promote malignant phenotypes. Fortunately, the conserved GYG/GFG signature motif in the pore domain provides a designated spot for mutational study in this regard.

Potassium channels in cell migration and metastasis

Metastasis causes >90% of human cancer deaths (Hanahan and Weinberg, 2011). The metastatic cascade is a multistep process that involves mobilization of primary tumor cells by migration, invasion into adjacent nontumoral regions, entry into the circulatory system, exit toward metastatic sites, and colonizing the secondary metastatic sites. As the first step, cell migration plays an integral role in metastasis and dictates the success of malignant dissemination. Migratory cells are polarized with mobile lamellipodia at the leading edge and contracting cell body at the trailing edge. Cell motility critically depends on dynamic volume regulation. There are local cell volume changes during cell migration: increases at the leading edge with lamellipodia protrusion and decreases at cell rear as it retracts (Fig. 3). The best-characterized functions of potassium channels in facilitating cell migration concern its capacity to cause volume changes by influencing potassium flow. An early study on transformed MDCK-F renal epithelial cell migration showed that specific

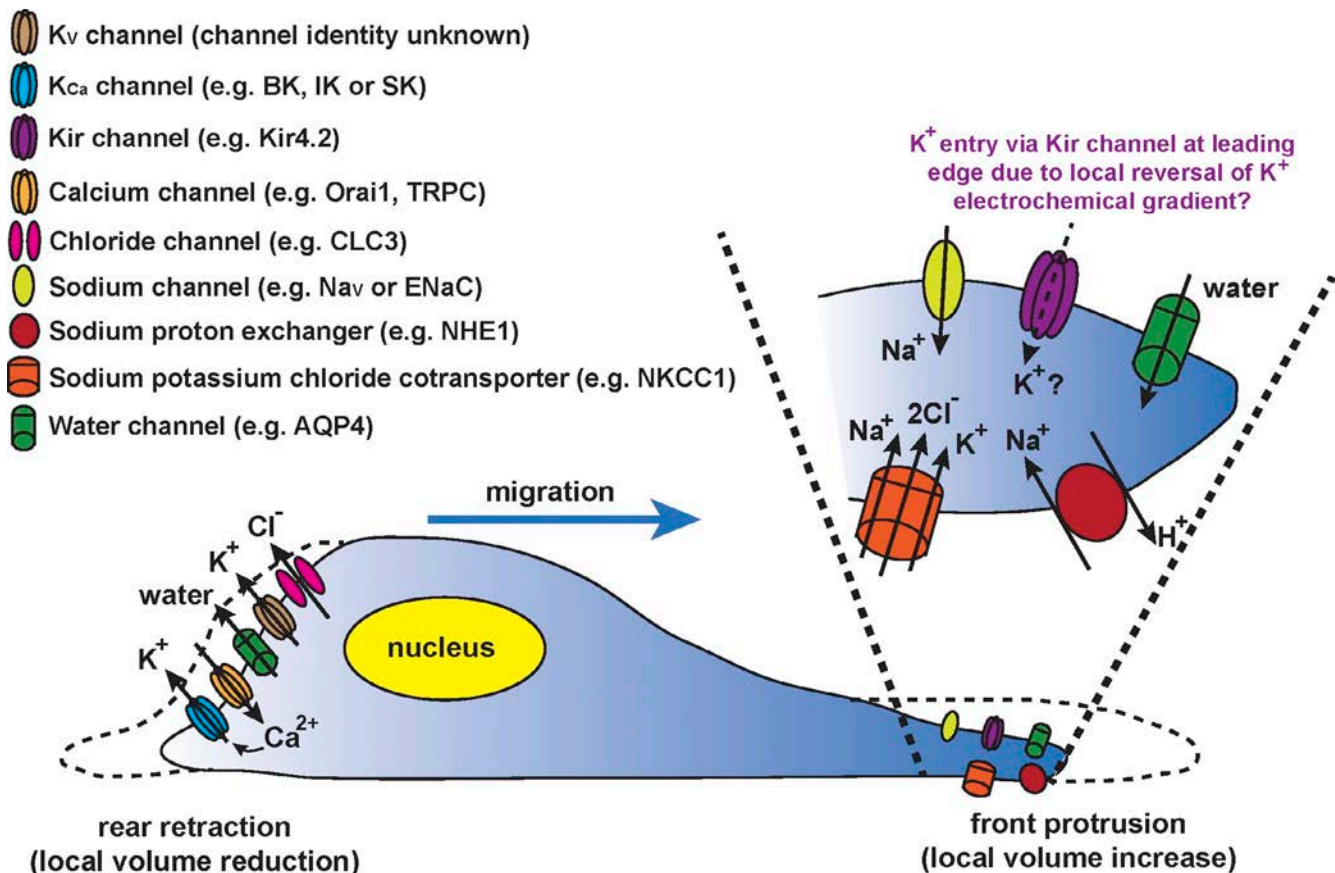


Figure 3. **Polarized ion and water flows drive local volume change and cell migration.** Cell migration is characterized by the polarized cellular and molecular processes that promote the protrusion of leading edge (local volume increase) and retraction of the trailing edge (local volume reduction). A net inflow of ions and water at the cell leading edge and a net outflow of ions and water at the cell trailing edge underlie these localized hydrodynamic changes. A number of ion channels and transporters display polarized subcellular distribution during cell migration. At the trailing edge, calcium entry through calcium-permeable channels can activate calcium-activated potassium channels to promote potassium efflux. The opening of chloride channels maintains electroneutrality and further enhances the outflow of ions, which is followed by obligatory water efflux through aquaporin. At the leading edge, an assortment of inward ion channels such as voltage-gated sodium channels (Nav) or epithelial sodium channels (ENaC), or ion transporters such as sodium-proton exchangers (NHE1) or sodium-potassium-chloride cotransporters (NKCC1) together with aquaporins, regulate ion and water influx. This ion and water influx drives local cell volume increase in the protruding lamellipodium for leading edge extension. The proton extrusion and acidification of extracellular microenvironment by NHE1 can further facilitate degradation of matrix proteins and tumor cell invasion (Brisson et al., 2011). Kir4.2 localizes at the cell leading edge and enhances cell migration (deHart et al., 2008). Whether Kir4.2 conducts an inward potassium flow, which will depend on the local electrochemical gradient for potassium at the leading edge, remains unclear. For comprehensive reviews on functional roles of the ion transportome during cell migration, see Cuddapah and Sontheimer (2011), Schwab et al. (2012), and Schwab and Stock (2014).

application of the IK channel blocker charybdotoxin to the trailing edge, but not the leading edge, inhibits migration and increases overall cell volume (Schwab et al., 1999; Schneider et al., 2000). However, IK calcium-activated potassium channels are enriched at the cell front (Schwab et al., 2006). One plausible scenario to account for these findings is that IK channels at the trailing edge may be preferentially activated, owing to a back-to-front calcium gradient in migrating cells (Schwab et al., 2012). It remains an open question whether potassium channels may be enriched at the trailing edge for local cell volume regulation (Fig. 3). The dependence on K_{Ca} channel activity in promoting cell migration has been repeatedly reported. For example, glioma cells exhibit a strong outward BK channel blocker-sensitive potassium current. Genetic suppression or pharmacological inhibition of BK channels impairs glioma cell migration (Weaver et al., 2006). Treating the mice bearing IK channel expressing glioblastoma with TRAM-34, a selective IK channel blocker, reduces tumor infiltration into brain parenchyma

(D'Alessandro et al., 2013). BK and SK channels can also promote breast cancer cell migration and are implicated in metastasis (Khaitan et al., 2009; Chantôme et al., 2013). The SK channel can form a complex with the Orai1 calcium channel for localized calcium entry within lipid rafts, providing a mechanism for its specific activation to enhance cancer cell migration (Chantôme et al., 2013). In addition, the BK channel can be functionally coupled with other ion channels, such as the CLC3 chloride channel, within the lipid rafts of invadopodia in glioma cells in order to mediate both potassium and chloride ion efflux, and to facilitate invasion by regulatory volume decrease to enable cells to course through a confined space (McFerrin and Sontheimer, 2006). Interestingly, chronic application of a BK channel opener also results in defective glioma cell migration (Kraft et al., 2003), demonstrating the importance of temporal and spatial control of potassium channel activity for coordinated cell movement. Recent studies have shown that IK channels modulate subventricular zone-derived neuroblast

migration along the rostral migratory stream to the olfactory bulb (Turner and Sontheimer, 2013), highlighting the function of potassium channels in regulating cell migration in both malignant and nonmalignant cell types. In addition to potassium channels, a variety of ion channels and transporters have been implicated in regulating local cell volume changes during cell migration (Fig. 3). Indeed, it has been recently shown that a net inflow of ions and water at the cell leading edge and outflow of ions and water at the cell trailing edge represents a major force for cell motility even in the absence of actin polymerization (Stroka et al., 2014).

Potassium channels can also regulate cancer cell migration via noncanonical functions. The best-illustrated example is the HERG channel, which can form a macromolecular complex with VEGFR-1 and $\beta 1$ integrin. The assembly of this protein complex confers a pro-migratory phenotype in leukemia, an effect that can be inhibited by application of a HERG channel blocker. Furthermore, HERG expression in leukemia patients is associated with higher probability of relapse and shorter survival (Pillozzi et al., 2007). It has been shown that colocalization of $\alpha 9\beta 1$ integrin with the Kir4.2 channel in focal adhesions at the leading edge of migrating cells may promote local Kir4.2 channel activity to induce the formation of a single dominant lamellipodial extension critical for persistent migration, although the underlying mechanism of how Kir4.2 modulates lamellipodium formation remains unclear (deHart et al., 2008; Fig. 3). These findings highlight the potassium channel as an integral membrane protein that is functionally coupled with other signaling molecules to exert concerted regulation on cell migration. Given the reported interactions between other potassium channels, focal adhesion molecules, and cytoskeleton proteins (Levite et al., 2000; Petrecca et al., 2000; Rezzonico et al., 2003), it is expected that future investigation will shed light on the novel noncanonical roles of potassium channels within the multiprotein complex during tumorigenic processes.

Therapeutic targeting of potassium channels in cancer

Being the most diverse class of ion channels that regulate a plethora of cellular behaviors in both excitable and nonexcitable cells, targeting the cell surface-accessible potassium channels offers tremendous therapeutic potential for the treatment of human diseases that involve aberrant expression and function of specific potassium channels. For example, drugs targeting K_{ATP} , KCNQ, and HERG channels have been successfully used in clinics for treating diabetes or hypertension, epilepsy, and cardiac arrhythmia, respectively. Despite the increasing evidence of ion channel functions in oncology, therapeutic development of cancer treatment using ion channel-targeting compounds is still at an early stage.

The most comprehensive cancer therapeutic efficacy study on potassium channels has focused on the EAG channel family, including EAG1, EAG2, and HERG. EAG1 overexpression confers tumorigenic potential, and its expression is correlated with poor patient survival in multiple cancer types (Pardo and Stühmer, 2008). Small molecule EAG1 blockers such as astemizole have shown efficacy in reducing EAG1-expressing cancer

cell growth *in vitro* and *in vivo* (Downie et al., 2008). Pharmacological inhibition of HERG channels reduces proliferation and impairs invasiveness in a variety of cancer cell types (Babcock and Li, 2013). Importantly, targeting the HERG channel with its specific blocker E-4031 impairs the function of the multiprotein complex consisting of HERG, $\beta 1$ integrin, and the chemokine receptor CXCR4, which is essential for leukemic cell survival. E-4031 treatment inhibits leukemia progression *in vivo* and enhances mouse survival (Pillozzi et al., 2011). Because the HERG channel mediates the repolarization phase of cardiac action potential and EAG channels share high structural similarity, one should be cautious about using these compounds for direct transition into treating human cancer patients, as HERG channel blockade can induce potentially fatal cardiac arrhythmia (long QT syndrome). This side effect has made the HERG channel an antitarget during modern drug development. Nevertheless, it should be noted that a number of marketed drugs treating various human conditions are known HERG inhibitors with well-characterized and acceptable toxicity profiles (Babcock et al., 2013). With careful treatment design and close monitoring of the potential adverse effects, these HERG-blocking drugs may be repurposed for cancer treatment. Generating monoclonal antibodies has proven to be an effective strategy to perturb the function of cell surface proteins. As a proof of principle, it has been demonstrated that a highly specific extracellular epitope-targeting EAG1 monoclonal antibody exhibited antitumor activity in mouse (Gómez-Varela et al., 2007). Given the fact that EAG1 is highly expressed in 70% of human cancers but minimally present in non-CNS normal tissues, dye-tagged EAG1 antibody also possesses the potential for selective visualization of tumor cells for diagnostic purposes (Pardo et al., 2005). EAG1-specific antibodies can be further coupled with cytotoxic agents for selective killing of EAG1-expressing cancer cells (Hartung et al., 2011).

Rational design of therapeutic targeting of potassium channels for cancer treatment will be based on our better understanding of their functions from mechanistic study. For example, cancer cells preferentially express an N-terminal truncated form of the HERG channel (Crociani et al., 2003), providing an opportunity for generating an isoform-specific antagonist. A recent study identified a lipid raft-localized SK3 potassium–Orai1 calcium channel complex that is essential to control breast cancer migration. Strikingly, the disruption of lipid rafts by the alkyl-lipid Ohmline leads to significantly reduced bone metastasis (Chantôme et al., 2013), highlighting the effectiveness of indirectly targeting potassium channel protein complexes that influence key processes of cancer progression. HERG channel protein maturation, stability, and current density can be enhanced by the stress-activated chaperone Sigma 1 receptor, which physically associates with HERG and represents a novel pharmacological target in a membrane signaling channel macrocomplex that is involved in cancer progression (Crottès et al., 2011). Potassium channel antagonism can also be exploited as a potential adjuvant therapy. HERG channel blockade reduces bone marrow mesenchymal stem cell-induced chemotherapeutic resistance in leukemic cells (Pillozzi et al., 2011). Ionizing radiation increases the open probability of BK channels without

affecting their expression levels and contributes to the enhanced migration and invasiveness of glioma cells (Steinle et al., 2011). Radiation treatment can also increase Kv3.4 channel activity in leukemic cells to promote calcium entry and CaMKII-dependent cancer cell survival (Palme et al., 2013). Low-dosage radiation induces voltage-dependent potassium channel activity that is otherwise not present in human lung adenocarcinoma cells (Kuo et al., 1993). These findings suggest that radiation-induced potassium channel activation may be a general response in different cancer types, and potassium channel inhibition may be a novel and promising component in combinatorial therapy for treating cancer patients.

Targeting potassium channels:

Unanswered questions

A growing body of evidence suggests that potassium channel dysregulation is associated with key aspects of cancer, and targeting potassium channel is a viable therapeutic approach. Different cancers may use specific channel types for their malignant behaviors, depending on the cellular origin of the tumor and the driving oncogenic signal. Since the identification of potassium channel involvement in T cell proliferation 30 years ago (DeCoursey et al., 1984), much progress has been made, yet research into potassium channel function in cancer still represents a nascent field. Significant advancement may come from future research into several important areas. First, there are many open questions regarding the possibility of heterogeneity in potassium channel expression or function in the cancer cell over space and time. For example, do cancer stem cells exploit different potassium channels for maintaining their unique properties such as enhanced tumorigenic potential and survival after irradiation and chemotherapy? Does a microenvironment such as hypoxia or cancer treatment induce differential potassium channel activity that confers drug resistance? Second, although the potassium channel expression level has been compared between tumor and nontumor tissues, the mutational landscape of potassium channels in various cancer types remains largely unexplored. The identification and quantification of recurrent mutations may offer another avenue to identify novel potassium channels involved in the oncogenic process, and stimulate the investigation of their functions. Third, the majority of studies reported thus far are based on in vitro cell culture systems and xenograft models. Future studies involving mutating potassium channels in the spontaneous cancer models established in various model organisms, such as fruit fly, zebrafish, and mouse, should reveal novel insights into potassium channel function during tumor initiation, progression, and evolution. Fourth, studying the therapeutic value of potassium channel-modulating drugs using genetic models of cancer should foster the rapid translation from preclinical discoveries to clinical trials. Fifth, advanced high-throughput screening technology should be exploited for identifying novel compounds that modulate potassium channels implicated in cancer, while significant efforts can also be made toward elucidating the therapeutic value of repurposing potassium channel-modulating drugs on the market for treating other human diseases. Although no current FDA-approved cancer drug is intended to function as a potassium

channel blocker, the future holds great promise for targeting potassium channels in cancer.

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