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# Membrane potential and cancer progression

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Membrane potential ( $V_m$ ), the voltage across the plasma membrane, arises because of the presence of different ion channels/transporters with specific ion selectivity and permeability.  $V_m$  is a key biophysical signal in non-excitable cells, modulating important cellular activities, such as proliferation and differentiation. Therefore, the multiplicities of various ion channels/transporters expressed on different cells are finely tuned in order to regulate the  $V_m$ . It is well-established that cancer cells possess distinct bioelectrical properties. Notably, electrophysiological analyses in many cancer cell types have revealed a depolarized  $V_m$  that favors cell proliferation. Ion channels/transporters control cell volume and migration, and emerging data also suggest that the level of  $V_m$  has functional roles in cancer cell migration. In addition, hyperpolarization is necessary for stem cell differentiation. For example, both osteogenesis and adipogenesis are hindered in human mesenchymal stem cells (hMSCs) under depolarizing conditions. Therefore, in the context of cancer, membrane depolarization might be important for the emergence and maintenance of cancer stem cells (CSCs), giving rise to sustained tumor growth. This review aims to provide a broad understanding of the  $V_m$  as a bioelectrical signal in cancer cells by examining several key types of ion channels that contribute to its regulation. The mechanisms by which  $V_m$  regulates cancer cell proliferation, migration, and differentiation will be discussed. In the long term,  $V_m$  might be a valuable clinical marker for tumor detection with prognostic value, and could even be artificially modified in order to inhibit tumor growth and metastasis.

**Keywords:** cancer, cell cycle, differentiation, ion channel, membrane potential, migration, proliferation, stem cell

## INTRODUCTION

The presence of various ion channels and transporters at the plasma membrane provides different permeability to distinct ions, such as  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Cl}^-$ . Due to the unequal distribution of these ions, a voltage difference exists between the cytoplasm and the extracellular environment, which is known as the membrane potential ( $V_m$ ).  $V_m$  is expressed relative to the extracellular environment. A cell is depolarized when the  $V_m$  is relatively less negative, whereas a hyperpolarized cell possesses a more negative  $V_m$ .  $V_m$  changes because of alterations in the conductance of one or more types of ion. The Goldman–Hodgkin–Katz equation shows that the  $V_m$  depends on the permeability ( $P$ ) and both the intracellular and extracellular concentrations of major ions (Goldman, 1943; Hodgkin and Katz, 1949):

$$V_m = \frac{RT}{F} \ln \left( \frac{P_{\text{Na}^+} [\text{Na}^+]_o + P_{\text{K}^+} [\text{K}^+]_o + P_{\text{Cl}^-} [\text{Cl}^-]_o}{P_{\text{Na}^+} [\text{Na}^+]_i + P_{\text{K}^+} [\text{K}^+]_i + P_{\text{Cl}^-} [\text{Cl}^-]_i} \right)$$

where  $R$  is the ideal gas constant,  $T$  the temperature, and  $F$  the Faraday constant. In addition, intercellular communications (e.g., gap junction connections) are also able to influence  $V_m$  (Hulser and Lauterwasser, 1982; Levin, 2007a). In excitable cells, such as neurons and muscle fibers (Nakajima and Horn, 1967; Bean, 2007), changes in  $V_m$  underlie the action potential (AP) waveform. APs fire in response to a depolarization that exceeds a

threshold value. Fine-tuning of APs is tightly regulated by the activities of several key ion channels and transporters, including voltage-gated  $\text{Na}^+$  channels (VGSCs), voltage-gated  $\text{K}^+$  channels ( $\text{K}_v$ ), and the  $\text{Na}^+/\text{K}^+$ -ATPase (Caldwell and Keynes, 1957; Hille, 1992).

Emerging evidence suggests that the  $V_m$  also plays important functional roles in non-excitable cells. In the late 1960's, while studying mitotic activities in sarcoma cells, Clarence D. Cone Jr. reported that  $V_m$  underwent hyperpolarization before entering M phase, and suggested that the level of  $V_m$  correlated with cell cycle progression (Cone, 1969). He subsequently showed that membrane hyperpolarization reversibly blocked DNA synthesis and mitosis (Cone, 1970). He later generalized existing data at that time and postulated that the  $V_m$  level was correlated with the level of differentiation. For example, terminally differentiated cells (e.g., fibroblasts and epithelium) possess hyperpolarized  $V_m$  (Cone, 1971). Since then, changes in  $V_m$ , representing the long-term, slowly changing bioelectric gradient in non-excitable cells (Lobikin et al., 2012), have been shown to control critical cell functions including proliferation, migration, and differentiation (Binggeli and Weinstein, 1986; Schwab et al., 2007; Blackiston et al., 2009; Sundelacruz et al., 2009). Recently, studies have also demonstrated that  $V_m$  is able to, directly or indirectly, control wound healing (Nuccitelli, 2003a,b; McCaig et al., 2009), left-right patterning (Adams et al., 2006), development (Nuccitelli, 2003a; Adams, 2008), and regeneration (Levin, 2007b,

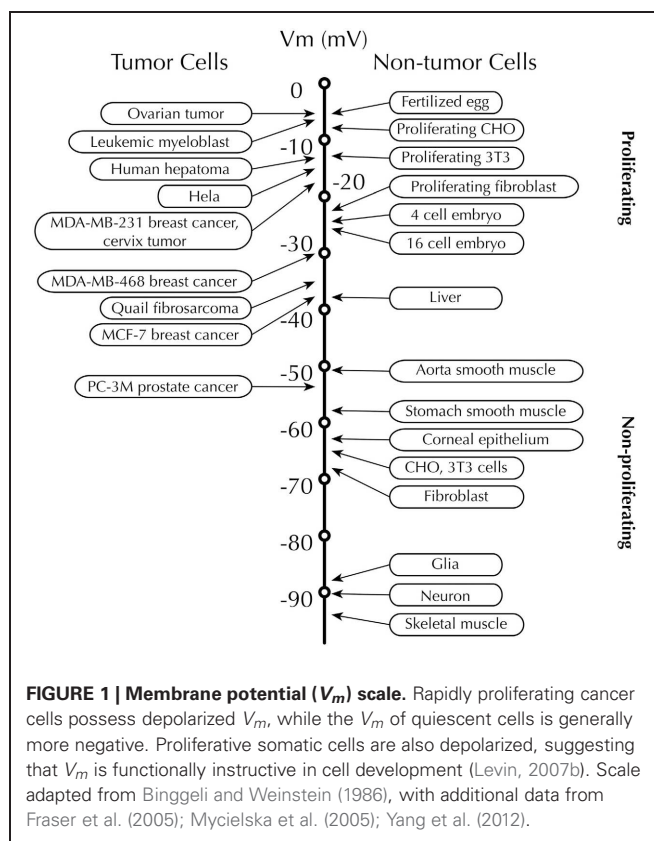
2009). Therefore, given the increasing evidence showing that ion channels/transporters functionally participate in cancer progression (Kunzelmann, 2005; Fiske et al., 2006; Stuhmer et al., 2006; Prevarskaya et al., 2010; Becchetti, 2011; Brackenbury, 2012), it is not surprising that  $V_m$  has been implicated in cancer development, since  $V_m$  is itself determined by the combined activities of ion channels/transporters at the cell membrane. This article aims to summarize current understanding of the  $V_m$  as a bioelectric regulator in cancer, and examines the therapeutic potential of  $V_m$  for tumor detection and treatment.

### CANCER CELLS POSSESS DEPOLARIZED $V_m$

Cone's theory proposing the general correlation between proliferation and  $V_m$  (Cone, 1971) was supported by several previous studies which demonstrated significant  $V_m$  depolarization during malignant transformation of normal cells (Tokuoka and Morioka, 1957; Johnstone, 1959). Direct *in vitro* and *in vivo* comparisons of  $V_m$  levels between normal and cancerous breast cells (Marino et al., 1994), hepatocytes and hepatocellular carcinoma cells (Binggeli and Cameron, 1980; Stevenson et al., 1989), normal and neoplastic adrenocortical tissues (Lymangrover et al., 1975), normal embryonic fibroblasts and fibrosarcoma (Binggeli and Weinstein, 1985), benign and cancerous skin cells (Melczer and Kiss, 1957; Woodrough et al., 1975), and between normal and cancerous ovarian tissue (Redmann et al., 1972) showed that cancer cells tended to be more depolarized than their normal counterparts. In addition, the intracellular  $\text{Na}^+$  level is markedly higher in tumors compared to non-cancerous tissues, whereas the  $\text{K}^+$  level remains more stable (Smith et al., 1978; Cameron et al., 1980; Sparks et al., 1983). A similar scenario occurs in fast proliferating Chinese hamster ovary (CHO) and 3T3 cells (Cone and Tongier, 1973). Thus, an increased intracellular  $\text{Na}^+$  concentration could be a determinant of a depolarized phenotype in rapidly cycling cancer cells.

Recordings from rodent and human tissues have revealed that proliferative cells, especially rapidly proliferating tumor cells, displayed depolarized  $V_m$ , whereas non-proliferating, terminally differentiated somatic cells, such as muscle cells and neurons, are characterized by their hyperpolarized  $V_m$  (Figure 1) [reviewed in Binggeli and Weinstein (1986)]. Given these findings, is  $V_m$  merely an epiphenomenon, which only indicates the outcome of the activities of various ion channels and transporters, or is it actually a functional instructor that is capable of promoting tumorigenesis? A similar question had been posed 50 years ago soon after Cone revealed the relationship between mitotic activity and  $V_m$  level (Cone and Tongier, 1971). For example, depolarization can initiate mitosis in CHO cells and mouse spleen lymphocytes (Cone and Tongier, 1971; Kiefer et al., 1980). By contrast, hyperpolarized  $V_m$  immediately precedes mitotic arrest (Cone and Tongier, 1973). More recently, *in vivo* evidence shows that membrane depolarization itself, regardless of the types of ions and ion channel/transporter proteins, is able to bring cancerous transformation (i.e., increased proliferation, change in morphology and abnormal angiogenesis) in *Xenopus laevis* embryos (Lobikin et al., 2012).

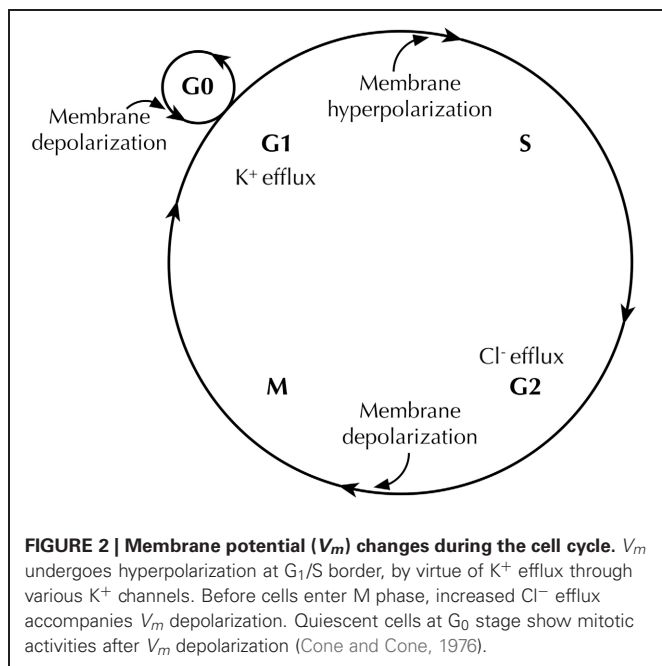
Hanahan and Weinberg proposed 10 hallmarks of cancer, including sustaining proliferative signaling, activating invasion



and metastasis, and angiogenesis (Hanahan and Weinberg, 2011). The following sections review the prevailing evidence that implicates  $V_m$  in several of these processes.

### $V_m$ AND CANCER CELL PROLIFERATION

In general, in both highly proliferative tumor and non-tumor cells, depolarization is believed to serve as a signal that could initiate mitosis and DNA synthesis (Orr et al., 1972; Binggeli and Weinstein, 1986). Artificially altering  $V_m$  by modulating the extracellular ionic constitution or applying the  $\text{Na}^+/\text{K}^+$ -ATPase inhibitor ouabain revealed interesting results: First, hyperpolarizing CHO cells to  $-45$  mV started to induce mitotic arrest and cell division was fully blocked at  $-75$  mV. The cell cycle was resumed by depolarizing the cells to  $-10$  mV (Cone, 1971). Secondly, quiescent ( $G_0$ ) mature chick spinal cord neurons showed mitotic activity after depolarization (Cone and Cone, 1976) (Figure 2). Recently, artificial control of  $V_m$  was accomplished in *Xenopus laevis* embryos by expressing glycine-gated  $\text{Cl}^-$  channels and applying the activator ivermectin. Depolarization (caused by lowering the  $\text{Cl}^-$  concentration in the extracellular medium, which caused  $\text{Cl}^-$  efflux) was found to be directly responsible for malignant proliferation. This proliferation was ion and ion channel non-specific, because (1) the phenotype caused by depolarization could be rescued by expressing a hyperpolarizing channel gene, and (2) the malignant phenotype could be induced or suppressed simply by adjusting extracellular  $\text{Cl}^-$  concentration, as predicted by Goldman-Hodgkin-Katz equation (Lobikin et al., 2012). Therefore, the depolarized  $V_m$  frequently found in



cancerous cell types could be regarded as a “sustaining proliferative signal” that instructs cells to rapidly advance in the cell cycle.

An additional layer of complexity in this model is that the  $V_m$  fluctuates during cell cycle progression, and follows a multi-step and rhythmic pattern (Wonderlin and Strobl, 1996; Blackiston et al., 2009) (Figure 2). A number of studies suggest that membrane hyperpolarization at the G<sub>1</sub>/S checkpoint is generally required for S phase initiation. For example, depolarizing the cell membrane halts G<sub>1</sub>/S progression in glia (Canady et al., 1990), Schwann cells (Wilson and Chiu, 1993), lymphocytes (Price et al., 1989; Freedman et al., 1992; Wang et al., 1992), V79 Chinese hamster lung cells (Sachs et al., 1974), C1300 mouse neuroblastoma cells (Boonstra et al., 1981), and MCF-7 human breast cancer cells (Wonderlin et al., 1995). The  $V_m$  then appears to remain relatively hyperpolarized through S phase in some cell types (Sachs et al., 1974; Boonstra et al., 1981; Strobl et al., 1995; Wonderlin et al., 1995), but is more depolarized in others (Arcangeli et al., 1995; Macfarlane and Sontheimer, 2000). The G<sub>2</sub>/M transition exhibits a depolarized  $V_m$  (Sachs et al., 1974; Boonstra et al., 1981; Blackiston et al., 2009), although it is not known whether or not this depolarization is a prerequisite for progression. In fact, the exact  $V_m$  thresholds for driving progression appear to depend heavily on cell type, the state of differentiation, and the density of cell monolayer in culture (Cone and Tongier, 1973; Blackiston et al., 2009).

Importantly, the fluctuation of  $V_m$  levels across the cell cycle does not necessarily contradict the observation that depolarized  $V_m$  could be a hallmark of cancer cells. The mean  $V_m$  values in cancer cells are consistently depolarized relative to most normal somatic cell types (Figure 1). For example, MCF-7 cells arrested at G<sub>1</sub> phase have a  $V_m$  of  $-9$  mV and hyperpolarize to  $\sim -30$  mV in the S phase (Wonderlin et al., 1995). Both

these values are more depolarized than normal breast cells, e.g., the mean  $V_m$  of unsynchronized MCF-10A cells is between  $-40$  and  $-58$  mV (Marino et al., 1994; Wonderlin et al., 1995; Fraser et al., 2005).

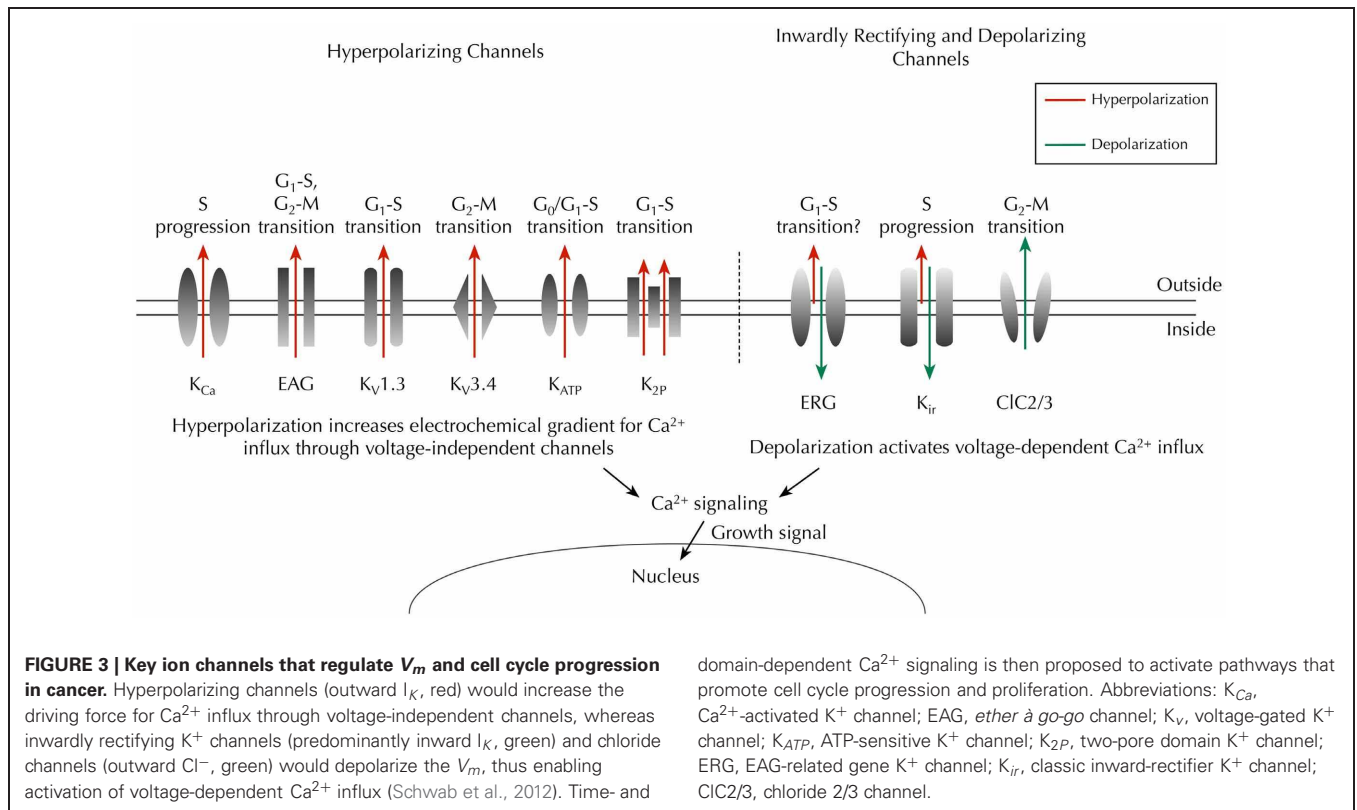
Evidence suggests that the fluctuation in K<sup>+</sup> concentration plays a significant contribution to changes in  $V_m$  during the cell cycle. For example, in neuroblastoma and Ehrlich ascites cells, there is a transient decrease in K<sup>+</sup> efflux before entering the G<sub>2</sub> phase, a relatively high level of K<sup>+</sup> efflux during the M phase (Mills and Tupper, 1976; Boonstra et al., 1981). Given the diversity of K<sup>+</sup> channel types (Hille, 1992; Miller, 2000; Wang, 2004), their relative contributions to the  $V_m$  and  $V_m$ -dependent cell cycle progression is probably context-dependent and highly complex. For example, inhibition of cell proliferation with K<sup>+</sup> channel inhibitors does not correlate with changes in the  $V_m$  in rat C6 glioma cells (Rouzaire-Dubois et al., 2000). In addition, the  $V_m$  is likely to be determined by the collective activities of a variety of ions/channels/transporters, which may exhibit reciprocal interactions and form a large and complex network responsible for  $V_m$  regulation and its downstream effects.

### ION CHANNEL-DEPENDENT REGULATION OF PROLIFERATION AND $V_m$

Numerous studies have shown that pharmacological or genetic block of K<sub>v</sub> channels reduces proliferation of cancer cells (e.g., Fraser et al., 2000; Ouadid-Ahidouch et al., 2000; Abdul and Hoosein, 2002; Chang et al., 2003; Menendez et al., 2010). Increasing evidence suggests that *Ether à go-go* (EAG) K<sup>+</sup> channels may serve as biomarkers for cancer (Ouadid-Ahidouch et al., 2001; Farias et al., 2004; Pardo et al., 2005; Hemmerlein et al., 2006; Ousingsawat et al., 2007; Ortiz et al., 2011; Rodriguez-Rasgado et al., 2012). Inhibition of EAG channel expression reduces proliferation in several cancer cell lines, whereas implantation of CHO cells over-expressing EAG channels in mice induces tumors (Pardo et al., 1999). In synchronized SH-SY5Y cells, human I<sub>EAG</sub> is reduced to less than 5% in G<sub>1</sub> phase, compared to unsynchronized controls, suggesting that the activity of EAG channels is cell cycle-dependent (Meyer and Heinemann, 1998). Indeed, in MCF-7 cells, inhibiting EAG channels with astemizole increases the proportion of cells in G<sub>1</sub> phase and reduces the proportion in S phase (Borowiec et al., 2007). In contrast, activation of hEAG channels is responsible for hyperpolarization at late G<sub>1</sub> before the cells enter the S phase (Ouadid-Ahidouch et al., 2001). Interestingly, the hyperpolarization is accompanied by increased Ca<sup>2+</sup>-activated K<sup>+</sup> (K<sub>Ca</sub>) channel currents (Ouadid-Ahidouch et al., 2001), which might result from the elevated intracellular Ca<sup>2+</sup> due to the increased electrochemical gradient (Figure 3) (Nilius and Wohlrab, 1992; Ouadid-Ahidouch and Ahidouch, 2008).

When K<sub>Ca</sub> channels were found in Friend murine erythroleukemia cells, they were thought to be one of the main controllers of the  $V_m$  (Arcangeli et al., 1987). K<sub>Ca</sub> channels have been found since in glioma (Liu et al., 2002), prostate cancer (Gessner et al., 2005), breast cancer (Haren et al., 2010), and the CD133<sup>+</sup> subpopulation of SH-SY5Y cells (Park et al., 2010). Inhibiting K<sub>Ca</sub> channels with iberiotoxin arrests D54-MG glioma cells in S phase, and leads to apoptosis (Weaver et al., 2004).





Thus, the functional contribution of  $K_{Ca}$  channels to cell cycle regulation appears to be distinct from  $K_V$  channels. In addition, in MCF-7 cells, inhibition of ATP-sensitive  $K^+$  ( $K_{ATP}$ ) channels reversibly arrests cells in the  $G_0/G_1$  phase (Woodfork et al., 1995). The two-pore domain  $K^+$  channel, TREK1, increases proliferation of PC-3 and LNCaP prostate cancer cells (Voloshyna et al., 2008). In CHO cells, overexpression of TREK1 increases the number of cells in S phase, and reduces the number of cells at  $G_0/G_1$  phase (Voloshyna et al., 2008).

Human EAG-related gene (HERG)  $K^+$  channels are strongly inwardly rectifying and conduct  $K^+$  influx when the voltage is more negative than the  $K^+$  equilibrium potential (Trudeau et al., 1995; Smith et al., 1996). HERG channels are expressed at early developmental stages in the neural crest, central nervous system, dorsal root ganglion (DRG) and skeletal muscle, and are replaced by classic inward rectifier  $K^+$  current ( $IK_{ir}$ ) later in development (Arcangeli et al., 1997; Crociani et al., 2000). HERG channels are upregulated in a number of cancers (Arcangeli, 2005). Moreover,  $I_{HERG}$  increases tumor cell proliferation (Bianchi et al., 1998; Wang et al., 2002). The activity of  $I_{HERG}$  itself is cell cycle dependent (Arcangeli et al., 1995), suggesting a complex relationship between  $I_{HERG}$ ,  $V_m$ , and proliferation. Additional inward rectifier  $K^+$  ( $K_{ir}$ ) channels have been reported in various cancer cell types, and are required for proliferation, including  $K_{ir2.2}$  (Lee et al., 2010),  $K_{ir3.1}$ , and  $K_{ir3.4}$  (Plummer et al., 2004; Takanami et al., 2004; Plummer et al., 2005; Wagner et al., 2010). In contrast, overexpression  $K_{ir4.1}$  in glioma cells hyperpolarizes the  $V_m$  and increases the number of cells in quiescent  $G_0/G_1$ , reducing the proportion in  $G_2/M$  phase (Higashimori and Sontheimer,

2007). Thus, different  $K_{ir}$  channels may play opposing roles in regulation of  $V_m$ /proliferation, as a result of their heterogeneous voltage dependence (Figure 3).  $Cl^-$  conductance also appears to be linked to the cell cycle and regulate proliferation. For example, in D54-MG cells,  $Cl^-$  efflux through the outward rectifying ClC3  $Cl^-$  channel is significantly increased during M phase (Habela et al., 2008). In addition, the ClC2 channel is expressed in M phase in transfected NRK-49F rat kidney fibroblast cells (Zheng et al., 2002).

The mechanisms underlying ion channel-dependent proliferation of cancer cells have been reviewed in detail elsewhere (Wang, 2004; Ouadid-Ahidouch and Ahidouch, 2008; Prevarskaya et al., 2010). These include possible non-conducting, direct interactions between ion channels and other pro-proliferative signaling mechanisms. For example, coexpression of HERG and tumor necrosis factor receptor 1 (TNFR1) has been found at the cell membrane of SKBR3 and SH-SY5Y cell lines, and HERG appears to recruit TNFR1 to the membrane, therefore enhancing TNF- $\alpha$ -induced cancer cell proliferation (Wang et al., 2002). Alternatively, ion channel-mediated  $V_m$  hyperpolarization would increase the electrochemical gradient for  $Ca^{2+}$  and therefore elevate the intracellular  $Ca^{2+}$  concentration through voltage-independent  $Ca^{2+}$  channels, such as transient receptor potential (TRP) channels (Nilius and Wohlrab, 1992; Wang, 2004; Ouadid-Ahidouch and Ahidouch, 2008).  $Ca^{2+}$  signaling is functional across the whole cell cycle (Santella et al., 2005). For example,  $Ca^{2+}$  is required for  $G_1$  progression and  $G_1/S$  transition (Hazelton et al., 1979; Choi et al., 2006). In turn, intracellular  $Ca^{2+}$  and calmodulin (CaM) can regulate

$K_{Ca}$  and EAG channels (Khanna et al., 1999; Ziechner et al., 2006; Ouadid-Ahidouch and Ahidouch, 2008). Thus, there may be a reciprocal, auto-regulatory relationship between ion channel activity,  $V_m$ , intracellular  $Ca^{2+}$  signaling, and proliferation.

In summary, a multiplicity of ion channels (predominantly  $K^+$ -conducting) participates in  $V_m$  regulation (both depolarization and hyperpolarization) in cancer cells. In turn, changes in  $V_m$  promote transition through cell cycle checkpoints. Changes in  $V_m$  are likely to trigger intracellular signaling messengers such as  $Ca^{2+}$  in order to drive sustained proliferation.

## ROLE OF $V_m$ IN CANCER CELL MIGRATION

Metastasis involves loss of adhesion at the primary site, increased migration and invasion, circulation through the vascular/lymphatic systems and growth of secondary tumors at distant sites (Gupta and Massague, 2006; Prevarskaya et al., 2010). Among the various steps in the metastatic cascade, it is well-established that cell migration is tightly controlled by the movement of ions and water [Figure 4; reviewed in depth in Schwab et al. (2007, 2012)].  $V_m$  is regarded as an indirect factor that can affect cell migration, whose main regulatory role might be setting up the electrical driving force for  $Ca^{2+}$  (Prevarskaya et al., 2010; Schwab et al., 2012). A hyperpolarized  $V_m$  can increase intracellular  $Ca^{2+}$  via TRP channels, whereas membrane depolarization could activate voltage-gated  $Ca^{2+}$  channels (Schwab et al., 2012). Intracellular  $Ca^{2+}$  displays a concentration gradient in migrating cells, with lowest concentration at the leading edge (Brundage et al., 1991). During cell migration, oscillations in  $Ca^{2+}$  concentration are observed within microdomains, such that  $Ca^{2+}$  flickering is highest in the lamellipodia (Wei et al., 2009). These fluctuations play a role in regulating tractional forces (Lee et al., 1999; Ridley et al., 2003), direction sensing, and cytoskeleton reorganization (Pettit and Fay, 1998).  $V_m$  may also affect downstream intracellular signaling cascades that could contribute

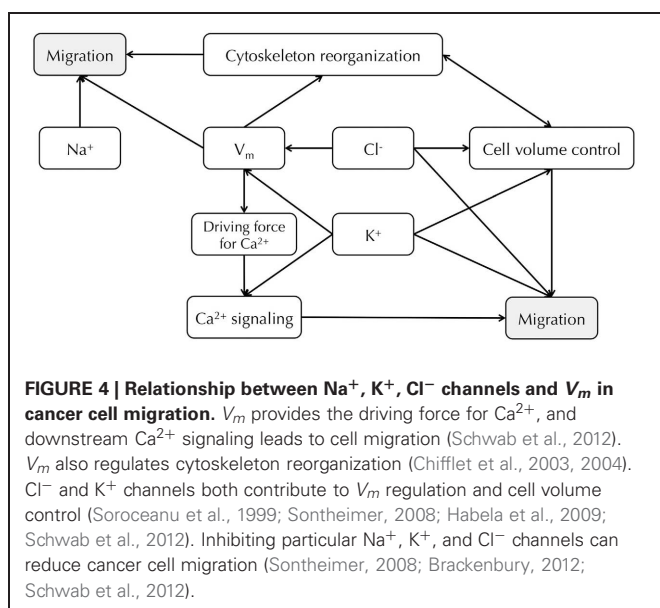
to cell migration in a  $Ca^{2+}$ -independent way (Figure 4). For example, in kidney epithelial cells,  $V_m$  depolarization induces diphosphorylation of myosin light chain (MLC) without inducing  $Ca^{2+}$  signaling, but instead by activating the Rho-Rho kinase (ROK) pathway (Szaszi et al., 2005). In addition, actin filaments undergo reorganization following  $V_m$  depolarization in bovine eye endothelial and epithelial cells (Chifflet et al., 2003, 2004), suggesting a functional role for  $V_m$  in cytoskeletal reorganization, although it is not clear whether or not  $Ca^{2+}$  is involved. Furthermore, applied electrical fields, which would impact on  $V_m$ , can enhance motility and galvanotaxis (Djamgoz et al., 2001; Levin, 2003, 2009; Schwab et al., 2012).

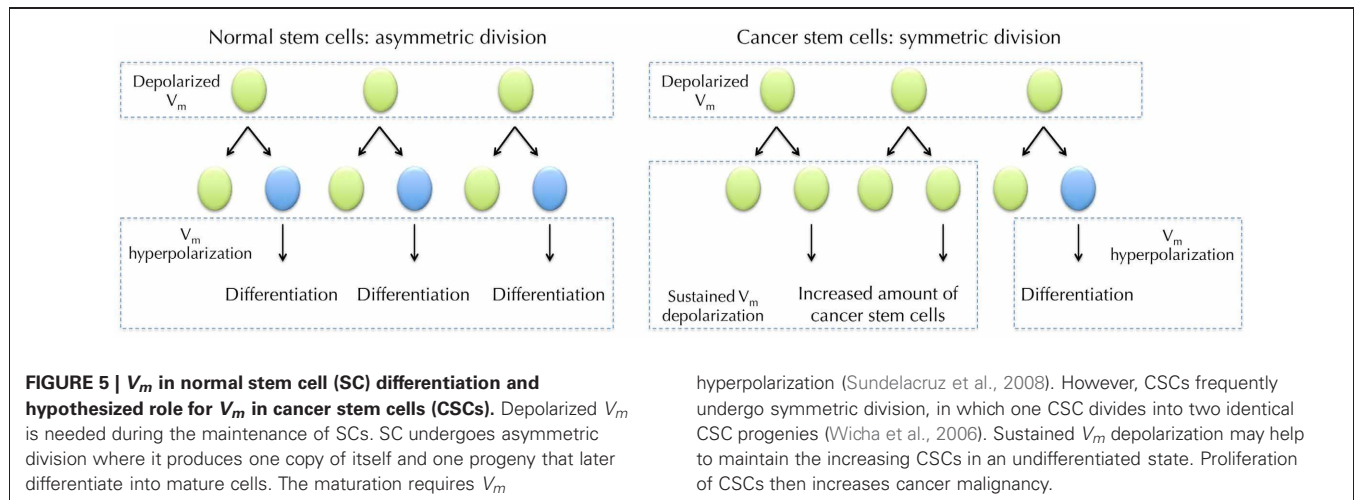
A number of  $Na^+$ ,  $K^+$ , and  $Cl^-$  channels, that potentially contribute to the  $V_m$ , are directly implicated in cancer cell migration. For example, functional VGSCs have been found in a number of cancer types [reviewed in Brackenbury (2012)], and suppressing VGSCs with siRNA or pharmacological agents inhibits migration and invasion (Roger et al., 2003; Fraser et al., 2005; Brackenbury et al., 2007; House et al., 2010; Yang et al., 2012). In several breast carcinoma/melanoma cell lines,  $K_{Ca2.3}$ , which is responsible for maintaining a hyperpolarized  $V_m$ , enhances migration, likely via promotion of intracellular  $Ca^{2+}$  signaling (Potier et al., 2006; Chantome et al., 2009). In addition,  $K_{Ca3.1}$  activity causes a local shrinkage at the rear of migrating MDCK-F cells, therefore supporting retraction at this pole during movement (Schwab et al., 2006). In order to maintain electroneutrality,  $K^+$  efflux must be accompanied by an anion, and  $Cl^-$  is the most likely candidate (Schwab et al., 2007, 2012). In agreement with this,  $Cl^-$  channels, which contribute to the depolarized  $V_m$  in glioma cells, enhance migration and invasion by permitting the release of  $K^+$ ,  $Cl^-$ , and water at the leading edge, resulting in shrinkage and facilitating movement into tortuous extracellular spaces (Soroceanu et al., 1999; Sontheimer, 2008; Habela et al., 2009; Schwab et al., 2012).

In conclusion, a direct role for  $V_m$  in regulating cancer cell migration is much less clear than for proliferation. Given the great variety of ion channels and transporters that are involved in the process of cell migration, the concept of the “transportome” has been proposed (Schwab et al., 2012), which implies that rather than individual ion channels or transporters, it is a complex network of ion translocators that directs the migration and invasion of cells (Figure 4). Further work is required to establish to what extent  $V_m$  directly impacts on this network.

## $V_m$ AND THE DIFFERENTIATION OF CANCER STEM CELLS

Stem cells and cancer cells share similar properties, such as the ability to differentiate and self-renew, increased membrane transporter activity and the ability to migrate and metastasize (Wicha et al., 2006). The cancer stem cell (CSC) hypothesis contains two key concepts: (1) cancers arise from dysregulated transformation of normal tissue stem cells or progenitor cells, and (2) cellular components that display stem cell properties can lead to cancer progression (Wicha et al., 2006). In contrast to normal, regulated asymmetric division of stem cells during tissue homeostasis, where a stem cell produces one copy of itself and one cell that later differentiates into a mature cell, the dysregulation of transformed CSCs during tumorigenesis involves “symmetric division” in





which each malign CSC generates two identical daughter cells (giving rise to either proliferation or differentiation), which significantly expands the malign stem cell reservoir (Figure 5) (Liu et al., 2005).

A role for  $V_m$  in differentiation of normal stem cells has been previously reported. Studies in quail neural crest cells and a subpopulation of SH-SY5Y cells have demonstrated that stem cells exhibit distinct bioelectrical profiles during development (Arcangeli et al., 1997; Biagiotti et al., 2006; Sundelacruz et al., 2009). In particular, a hyperpolarized  $V_m$  is required during stem cell maturation (Sundelacruz et al., 2009). For example,  $K_{ir}$ -induced  $V_m$  hyperpolarization is required during human myoblast fusion (Liu et al., 1998). In a genome-wide microarray analysis of depolarization-regulated genes in postnatal mouse cerebellar granule neurons, among 87 depolarization-responsive genes, 22 are developmentally up-regulated and 26 are developmentally down-regulated (Sato et al., 2005). Remarkably, 18 of the 22 (82%) developmentally up-regulated genes coincide with depolarization down-regulated genes, and 20 of 26 (77%) developmentally down-regulated genes with depolarization up-regulated genes (Sato et al., 2005).  $V_m$  hyperpolarization is also a functional determinant of human mesenchymal stem cell (hMSC) differentiation. Pharmacologically-induced  $V_m$  depolarization suppresses adipogenic and osteogenic differentiation of hMSCs (Sundelacruz et al., 2008). In addition, depolarization reduces the differentiated phenotype of hMSC-derived cells and improves their ability to transdifferentiate, without fully restoring a stem cell-like genetic profile (Sundelacruz et al., 2013). Taken together, these data suggest that  $V_m$  depolarization may

maintain cells in an undifferentiated stage at the gene expression level. Therefore, it is not unreasonable to postulate that depolarized  $V_m$  may also help maintain a population of undifferentiated CSCs (Figure 5). This possibility would raise additional, related questions: does a more depolarized  $V_m$  promote the proliferation of CSCs? Does  $V_m$  affect the pattern of symmetric vs. asymmetric division? Further work is required to investigate these possibilities.

## CLINICAL IMPLICATIONS

Given that the fluctuation of  $V_m$  can functionally regulate tumorigenesis, differentiation, and promote cancer progression, it may serve as a potential marker for tumor detection and treatment, with prognostic value. For example, bioelectrical impedance analysis, which determines tissue electrical properties, has shown promise as a prognostic indicator to monitor cancer progression (Gupta et al., 2004a,b), and recently, the development of non-invasive, voltage-sensitive optical probes provides a potential approach for *in vivo*  $V_m$  measurement (Adams and Levin, 2012; Chernet and Levin, 2013). Considering the vast array of therapeutic drugs that target ion channels (Sontheimer, 2008; Stuhmer and Pardo, 2010; D'amico et al., 2013; Djamgoz and Onkal, 2013), modulating the  $V_m$  of malign tissues by adjusting the activities of various ion channels/transporters may provide a convenient clinical approach.

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## REFERENCES

- Abdul, M., and Hoosain, N. (2002). Expression and activity of potassium ion channels in human prostate cancer. *Cancer Lett.* 186, 99–105. doi: 10.1016/S0304-3835(02)00348-8
- Adams, D. S. (2008). A new tool for tissue engineers: ions as regulators of morphogenesis during development and regeneration. *Tissue Eng. Part A* 14, 1461–1468. doi: 10.1089/ten.tea.2008.0080
- Adams, D. S., and Levin, M. (2012). General principles for measuring resting membrane potential and ion concentration using fluorescent bioelectricity reporters. *Cold Spring Harb. Protoc.* 2012, 385–397.
- Adams, D. S., Robinson, K. R., Fukumoto, T., Yuan, S., Albertson, R. C., Yelick, P., et al. (2006). Early,  $H^+$ - $V$ -ATPase-dependent proton flux is necessary for consistent left-right patterning of non-mammalian vertebrates. *Development* 133, 1657–1671. doi: 10.1242/dev.02341
- Arcangeli, A. (2005). Expression and role of hERG channels in cancer cells. *Novartis Found. Symp.* 266, 225–232. discussion: 232–234. doi: 10.1002/047002142X.ch17
- Arcangeli, A., Bianchi, L., Becchetti, A., Faravelli, L., Coronello, M., Mini, E., et al. (1995). A novel inward-rectifying  $K^+$  current with a cell-cycle dependence governs

- the resting potential of mammalian neuroblastoma cells. *J. Physiol.* 489(Pt 2), 455–471.
- Arcangeli, A., Rosati, B., Cherubini, A., Crociani, O., Fontana, L., Ziller, C., et al. (1997). HERG- and IRK-like inward rectifier currents are sequentially expressed during neuronal development of neural crest cells and their derivatives. *Eur. J. Neurosci.* 9, 2596–2604. doi: 10.1111/j.1460-9568.1997.tb01689.x
- Arcangeli, A., Wanke, E., Olivetto, M., Camagni, S., and Ferroni, A. (1987). Three types of ion channels are present on the plasma membrane of Friend erythroleukemia cells. *Biochem. Biophys. Res. Commun.* 146, 1450–1457. doi: 10.1016/0006-291X(87)90812-6
- Bean, B. P. (2007). The action potential in mammalian central neurons. *Nat. Rev. Neurosci.* 8, 451–465. doi: 10.1038/nrn2148
- Beccetti, A. (2011). Ion channels and transporters in cancer. 1. Ion channels and cell proliferation in cancer. *Am. J. Physiol. Cell Physiol.* 301, C255–C265. doi: 10.1152/ajpcell.00047.2011
- Biagiotti, T., D'Amico, M., Marzi, I., Di Gennaro, P., Arcangeli, A., Wanke, E., et al. (2006). Cell renewing in neuroblastoma: electrophysiological and immunocytochemical characterization of stem cells and derivatives. *Stem Cells* 24, 443–453. doi: 10.1634/stemcells.2004-0264
- Bianchi, L., Wible, B., Arcangeli, A., Tagliatalata, M., Morra, F., Castaldo, P., et al. (1998). hERG encodes a K<sup>+</sup> current highly conserved in tumors of different histogenesis: a selective advantage for cancer cells? *Cancer Res.* 58, 815–822.
- Binggeli, R., and Cameron, I. L. (1980). Cellular potentials of normal and cancerous fibroblasts and hepatocytes. *Cancer Res.* 40, 1830–1835.
- Binggeli, R., and Weinstein, R. C. (1985). Deficits in elevating membrane potential of rat fibrosarcoma cells after cell contact. *Cancer Res.* 45, 235–241.
- Binggeli, R., and Weinstein, R. C. (1986). Membrane potentials and sodium channels: hypotheses for growth regulation and cancer formation based on changes in sodium channels and gap junctions. *J. Theor. Biol.* 123, 377–401. doi: 10.1016/S0022-5193(86)80209-0
- Blackiston, D. J., McLaughlin, K. A., and Levin, M. (2009). Bioelectric controls of cell proliferation: ion channels, membrane voltage and the cell cycle. *Cell Cycle* 8, 3519–3528. doi: 10.4161/cc.8.21.9888
- Boonstra, J., Mummery, C. L., Tertoolen, L. G., Van Der Saag, P. T., and De Laat, S. W. (1981). Cation transport and growth regulation in neuroblastoma cells. Modulations of K<sup>+</sup> transport and electrical membrane properties during the cell cycle. *J. Cell. Physiol.* 107, 75–83. doi: 10.1002/jcp.1041070110
- Borowiec, A. S., Hague, F., Harir, N., Guenin, S., Guerin, F., Gouilleux, F., et al. (2007). IGF-1 activates hEAG K(+) channels through an Akt-dependent signaling pathway in breast cancer cells: role in cell proliferation. *J. Cell. Physiol.* 212, 690–701. doi: 10.1002/jcp.21065
- Brackenbury, W. J. (2012). Voltage-gated sodium channels and metastatic disease. *Channels (Austin)* 6, 352–361. doi: 10.4161/chan.21910
- Brackenbury, W. J., Chioni, A. M., Diss, J. K., and Djamgoz, M. B. (2007). The neonatal splice variant of Nav1.5 potentiates *in vitro* invasive behaviour of MDA-MB-231 human breast cancer cells. *Breast Cancer Res. Treat.* 101, 149–160. doi: 10.1007/s10549-006-9281-1
- Brundage, R. A., Fogarty, K. E., Tuft, R. A., and Fay, F. S. (1991). Calcium gradients underlying polarization and chemotaxis of eosinophils. *Science* 254, 703–706. doi: 10.1126/science.1948048
- Caldwell, P. C., and Keynes, R. D. (1957). The utilization of phosphate bond energy for sodium extrusion from giant axons. *J. Physiol.* 137, 12P–13P.
- Cameron, I. L., Smith, N. K., Pool, T. B., and Sparks, R. L. (1980). Intracellular concentration of sodium and other elements as related to mitogenesis and oncogenesis *in vivo*. *Cancer Res.* 40, 1493–1500.
- Canady, K. S., Ali-Osman, F., and Rubel, E. W. (1990). Extracellular potassium influences DNA and protein syntheses and glial fibrillary acidic protein expression in cultured glial cells. *Glia* 3, 368–374. doi: 10.1002/glia.440030508
- Chang, K. W., Yuan, T. C., Fang, K. P., Yang, F. S., Liu, C. J., Chang, C. S., et al. (2003). The increase of voltage-gated potassium channel Kv3.4 mRNA expression in oral squamous cell carcinoma. *J. Oral Pathol. Med.* 32, 606–611. doi: 10.1034/j.1600-0714.2003.00197.x
- Chantome, A., Girault, A., Potier, M., Collin, C., Vaudin, P., Pages, J. C., et al. (2009). KCa2.3 channel-dependent hyperpolarization increases melanoma cell motility. *Exp. Cell Res.* 315, 3620–3630. doi: 10.1016/j.yexcr.2009.07.021
- Chernet, B. T., and Levin, M. (2013). Transmembrane voltage potential is an essential cellular parameter for the detection and control of tumor development in a *Xenopus* model. *Dis. Model. Mech.* 6, 595–607. doi: 10.1242/dmm.010835
- Chifflet, S., Correa, V., Nin, V., Justet, C., and Hernandez, J. A. (2004). Effect of membrane potential depolarization on the organization of the actin cytoskeleton of eye epithelia. The role of adherens junctions. *Exp. Eye Res.* 79, 769–777. doi: 10.1016/j.exer.2004.08.031
- Chifflet, S., Hernandez, J. A., Grasso, S., and Cirillo, A. (2003). Nonspecific depolarization of the plasma membrane potential induces cytoskeletal modifications of bovine corneal endothelial cells in culture. *Exp. Cell Res.* 282, 1–13. doi: 10.1006/excr.2002.5664
- Choi, J., Chiang, A., Taulier, N., Gros, R., Pirani, A., and Husain, M. (2006). A calmodulin-binding site on cyclin E mediates Ca<sup>2+</sup>-sensitive G1/s transitions in vascular smooth muscle cells. *Circ. Res.* 98, 1273–1281. doi: 10.1161/01.RES.0000223059.19250.91
- Cone, C. D. Jr. (1969). Electroosmotic interactions accompanying mitosis initiation in sarcoma cells *in vitro*. *Trans. N.Y. Acad. Sci.* 31, 404–427. doi: 10.1111/j.2164-0947.1969.tb02926.x
- Cone, C. D. Jr. (1970). Variation of the transmembrane potential level as a basic mechanism of mitosis control. *Oncology* 24, 438–470. doi: 10.1159/000224545
- Cone, C. D. Jr. (1971). Unified theory on the basic mechanism of normal mitotic control and oncogenesis. *J. Theor. Biol.* 30, 151–181. doi: 10.1016/0022-5193(71)90042-7
- Cone, C. D. Jr., and Cone, C. M. (1976). Induction of mitosis in mature neurons in central nervous system by sustained depolarization. *Science* 192, 155–158. doi: 10.1126/science.56781
- Cone, C. D. Jr., and Tongier, M. Jr. (1971). Control of somatic cell mitosis by simulated changes in the transmembrane potential level. *Oncology* 25, 168–182. doi: 10.1159/000224567
- Cone, C. D. Jr., and Tongier, M. Jr. (1973). Contact inhibition of division: involvement of the electrical transmembrane potential. *J. Cell. Physiol.* 82, 373–386. doi: 10.1002/jcp.1040820307
- Crociani, O., Cherubini, A., Piccini, E., Polvani, S., Costa, L., Fontana, L., et al. (2000). *erg* gene(s) expression during development of the nervous and muscular system of quail embryos. *Mech. Dev.* 95, 239–243. doi: 10.1016/S0925-4773(00)00335-X
- D'Amico, M., Gasparoli, L., and Arcangeli, A. (2013). Potassium channels: novel emerging biomarkers and targets for therapy in cancer. *Recent Pat. Anticancer Drug Discov.* 8, 53–65.
- Djamgoz, M. B., and Onkal, R. (2013). Persistent current blockers of voltage-gated sodium channels: a clinical opportunity for controlling metastatic disease. *Recent Pat. Anticancer Drug Discov.* 8, 66–84.
- Djamgoz, M. B. A., Mycielska, M., Madeja, Z., Fraser, S. P., and Korohoda, W. (2001). Directional movement of rat prostate cancer cells in direct-current electric field: involvement of voltage gated Na<sup>+</sup> channel activity. *J. Cell Sci.* 114, 2697–2705.
- Farias, L. M., Ocana, D. B., Diaz, L., Larrea, F., Avila-Chavez, E., Cadena, A., et al. (2004). Ether a go-go potassium channels as human cervical cancer markers. *Cancer Res.* 64, 6996–7001. doi: 10.1158/0008-5472.CAN-04-1204
- Fiske, J. L., Fomin, V. P., Brown, M. L., Duncan, R. L., and Sikes, R. A. (2006). Voltage-sensitive ion channels and cancer. *Cancer Metastasis Rev.* 25, 493–500. doi: 10.1007/s10555-006-9017-z
- Fraser, S. P., Diss, J. K., Chioni, A. M., Mycielska, M. E., Pan, H., Yamaci, R. F., et al. (2005). Voltage-gated sodium channel expression and potentiation of human breast cancer metastasis. *Clin. Cancer Res.* 11, 5381–5389. doi: 10.1158/1078-0432.CCR-05-0327
- Fraser, S. P., Grimes, J. A., and Djamgoz, M. B. (2000). Effects of voltage-gated ion channel modulators on rat prostatic cancer cell proliferation: comparison of strongly and weakly metastatic cell lines. *Prostate* 44, 61–76.
- Freedman, B. D., Price, M. A., and Deutsch, C. J. (1992). Evidence for voltage modulation of IL-2 production in mitogen-stimulated human peripheral blood lymphocytes. *J. Immunol.* 149, 3784–3794.
- Gessner, G., Schonherr, K., Soom, M., Hansel, A., Asim, M., Baniahmad, A., et al. (2005). BKCa channels activating at resting potential without calcium in LNCaP prostate cancer



- cells. *J. Membr. Biol.* 208, 229–240. doi: 10.1007/s00232-005-0830-z
- Goldman, D. E. (1943). Potential, impedance, and rectification in membranes. *J. Gen. Physiol.* 27, 37–60. doi: 10.1085/jgp.27.1.37
- Gupta, D., Lammersfeld, C. A., Burrows, J. L., Dahlk, S. L., Vashi, P. G., Grutsch, J. F., et al. (2004a). Bioelectrical impedance phase angle in clinical practice: implications for prognosis in advanced colorectal cancer. *Am. J. Clin. Nutr.* 80, 1634–1638.
- Gupta, D., Lis, C. G., Dahlk, S. L., Vashi, P. G., Grutsch, J. F., and Lammersfeld, C. A. (2004b). Bioelectrical impedance phase angle as a prognostic indicator in advanced pancreatic cancer. *Br. J. Nutr.* 92, 957–962. doi: 10.1079/BJN20041292
- Gupta, G. P., and Massague, J. (2006). Cancer metastasis: building a framework. *Cell* 127, 679–695. doi: 10.1016/j.cell.2006.11.001
- Habela, C. W., Ernest, N. J., Swindall, A. F., and Sontheimer, H. (2009). Chloride accumulation drives volume dynamics underlying cell proliferation and migration. *J. Neurophysiol.* 101, 750–757. doi: 10.1152/jn.90840.2008
- Habela, C. W., Olsen, M. L., and Sontheimer, H. (2008). CIC3 is a critical regulator of the cell cycle in normal and malignant glial cells. *J. Neurosci.* 28, 9205–9217. doi: 10.1523/JNEUROSCI.1897-08.2008
- Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell* 144, 646–674. doi: 10.1016/j.cell.2011.02.013
- Haren, N., Khorsi, H., Faouzi, M., Ahidouch, A., Sevestre, H., and Ouadid-Ahidouch, H. (2010). Intermediate conductance Ca<sup>2+</sup> activated K<sup>+</sup> channels are expressed and functional in breast adenocarcinomas: correlation with tumour grade and metastasis status. *Histol. Histopathol.* 25, 1247–1255.
- Hazelton, B., Mitchell, B., and Tupper, J. (1979). Calcium, magnesium, and growth control in the WI-38 human fibroblast cell. *J. Cell Biol.* 83, 487–498. doi: 10.1083/jcb.83.2.487
- Hemmerlein, B., Weseloh, R. M., Mello De Queiroz, F., Knotgen, H., Sanchez, A., Rubio, M. E., et al. (2006). Overexpression of Eag1 potassium channels in clinical tumours. *Mol. Cancer* 5:41. doi: 10.1186/1476-4598-5-41
- Higashimori, H., and Sontheimer, H. (2007). Role of Kir4.1 channels in growth control of glia. *Glia* 55, 1668–1679. doi: 10.1002/glia.20574
- Hille, B. (1992). *Ionic Channels of Excitable Membranes*. Sunderland, MA: Sinauer Associates.
- Hodgkin, A. L., and Katz, B. (1949). The effect of sodium ions on the electrical activity of giant axon of the squid. *J. Physiol.* 108, 37–77.
- House, C. D., Vaske, C. J., Schwartz, A. M., Obias, V., Frank, B., Luu, T., et al. (2010). Voltage-gated Na<sup>+</sup> channel SCN5A is a key regulator of a gene transcriptional network that controls colon cancer invasion. *Cancer Res.* 70, 6957–6967. doi: 10.1158/0008-5472.CAN-10-1169
- Hulser, D. F., and Lauterwasser, U. (1982). Membrane potential oscillations in homokaryons. An endogenous signal for detecting intercellular communication. *Exp. Cell Res.* 139, 63–70. doi: 10.1016/0014-4827(82)90318-4
- Johnstone, B. M. (1959). Micro-electrode penetration of ascites tumour cells. *Nature* 183, 411. doi: 10.1038/183411a0
- Khanna, R., Chang, M. C., Joiner, W. J., Kaczmarek, L. K., and Schlichter, L. C. (1999). hSK4/hIK1, a calmodulin-binding K<sup>+</sup> channel in human T lymphocytes. Roles in proliferation and volume regulation. *J. Biol. Chem.* 274, 14838–14849. doi: 10.1074/jbc.274.21.14838
- Kiefer, H., Blume, A. J., and Kaback, H. R. (1980). Membrane potential changes during mitogenic stimulation of mouse spleen lymphocytes. *Proc. Natl. Acad. Sci. U.S.A.* 77, 2200–2204. doi: 10.1073/pnas.77.4.2200
- Kunzelmann, K. (2005). Ion channels and cancer. *J. Membr. Biol.* 205, 159–173. doi: 10.1007/s00232-005-0781-4
- Lee, I., Park, C., and Kang, W. K. (2010). Knockdown of inwardly rectifying potassium channel Kir2.2 suppresses tumorigenesis by inducing reactive oxygen species-mediated cellular senescence. *Mol. Cancer Ther.* 9, 2951–2959. doi: 10.1158/1535-7163.MCT-10-0511
- Lee, J., Ishihara, A., Oxford, G., Johnson, B., and Jacobson, K. (1999). Regulation of cell movement is mediated by stretch-activated calcium channels. *Nature* 400, 382–386. doi: 10.1038/22578
- Levin, M. (2003). Bioelectromagnetics in morphogenesis. *Bioelectromagnetics* 24, 295–315. doi: 10.1002/bem.10104
- Levin, M. (2007a). Gap junctional communication in morphogenesis. *Prog. Biophys. Mol. Biol.* 94, 186–206. doi: 10.1016/j.pbiomolbio.2007.03.005
- Levin, M. (2007b). Large-scale biophysics: ion flows and regeneration. *Trends Cell Biol.* 17, 261–270. doi: 10.1016/j.tcb.2007.04.007
- Levin, M. (2009). Bioelectric mechanisms in regeneration: unique aspects and future perspectives. *Semin. Cell Dev. Biol.* 20, 543–556. doi: 10.1016/j.semcdb.2009.04.013
- Liu, J. H., Bijlenga, P., Fischer-Lougheed, J., Occhiodoro, T., Kaelin, A., Bader, C. R., et al. (1998). Role of an inward rectifier K<sup>+</sup> current and of hyperpolarization in human myoblast fusion. *J. Physiol.* 510(Pt 2), 467–476. doi: 10.1111/j.1469-7793.1998.467bk.x
- Liu, S., Dontu, G., and Wicha, M. S. (2005). Mammary stem cells, self-renewal pathways, and carcinogenesis. *Breast Cancer Res.* 7, 86–95. doi: 10.1186/bcr1021
- Liu, X., Chang, Y., Reinhart, P. H., Sontheimer, H., and Chang, Y. (2002). Cloning and characterization of glioma BK, a novel BK channel isoform highly expressed in human glioma cells. *J. Neurosci.* 22, 1840–1849.
- Lobikin, M., Chernet, B., Lobo, D., and Levin, M. (2012). Resting potential, oncogene-induced tumorigenesis, and metastasis: the bioelectric basis of cancer *in vivo*. *Phys. Biol.* 9:065002. doi: 10.1088/1478-3975/9/6/065002
- Lymangrover, J., Pearlmutter, A. F., Franco-Saenz, R., and Saffran, M. (1975). Transmembrane potentials and steroidogenesis in normal and neoplastic human adrenocortical tissue. *J. Clin. Endocrinol. Metab.* 41, 697–706. doi: 10.1210/jcem-41-4-697
- Macfarlane, S. N., and Sontheimer, H. (2000). Changes in ion channel expression accompany cell cycle progression of spinal cord astrocytes. *Glia* 30, 39–48.
- Marino, A. A., Morris, D. M., Schwalke, M. A., Iliev, I. G., and Rogers, S. (1994). Electrical potential measurements in human breast cancer and benign lesions. *Tumour Biol.* 15, 147–152. doi: 10.1159/000217885
- McCaig, C. D., Song, B., and Rajnicek, A. M. (2009). Electrical dimensions in cell science. *J. Cell Sci.* 122, 4267–4276. doi: 10.1242/jcs.023564
- Melcer, N., and Kiss, J. (1957). Electrical method for detection of early cancerous growth of the skin. *Nature* 179, 1177–1179. doi: 10.1038/1791177b0
- Menendez, S. T., Rodrigo, J. P., Allonca, E., Garcia-Carracedo, D., Alvarez-Alija, G., Casado-Zapico, S., et al. (2010). Expression and clinical significance of the Kv3.4 potassium channel subunit in the development and progression of head and neck squamous cell carcinomas. *J. Pathol.* 221, 402–410.
- Meyer, R., and Heinemann, S. H. (1998). Characterization of an eag-like potassium channel in human neuroblastoma cells. *J. Physiol.* 508(Pt 1), 49–56.
- Miller, C. (2000). An overview of the potassium channel family. *Genome Biol.* 1:REVIEWS0004. doi: 10.1186/gb-2000-1-4-reviews0004
- Mills, B., and Tupper, J. T. (1976). Cell cycle dependent changes in potassium transport. *J. Cell. Physiol.* 89, 123–132. doi: 10.1002/jcp.1040890112
- Mycielska, M. E., Palmer, C. P., Brackenbury, W. J., and Djamgoz, M. B. (2005). Expression of Na<sup>+</sup>-dependent citrate transport in a strongly metastatic human prostate cancer PC-3M cell line: regulation by voltage-gated Na<sup>+</sup> channel activity. *J. Physiol.* 563, 393–408. doi: 10.1113/jphysiol.2004.079491
- Nakajima, A., and Horn, L. (1967). Electrical activity of single vascular smooth muscle fibers. *Am. J. Physiol.* 213, 25–30.
- Niluis, B., and Wohlrab, W. (1992). Potassium channels and regulation of proliferation of human melanoma cells. *J. Physiol.* 445, 537–548.
- Nuccitelli, R. (2003a). Endogenous electric fields in embryos during development, regeneration and wound healing. *Radiat. Prot. Dosimetry* 106, 375–383. doi: 10.1093/oxfordjournals.rpd.a006375
- Nuccitelli, R. (2003b). A role for endogenous electric fields in wound healing. *Curr. Top. Dev. Biol.* 58, 1–26. doi: 10.1016/S0070-2153(03)58001-2
- Orr, C. W., Yoshikawa-Fukada, M., and Ebert, J. D. (1972). Potassium: effect on DNA synthesis and multiplication of baby-hamster kidney cells: (cell cycle-membrane potential-synchronization-transformation). *Proc. Natl. Acad. Sci. U.S.A.* 69, 243–247. doi: 10.1073/pnas.69.1.243
- Ortiz, C. S., Montante-Montes, D., Saqui-Salces, M., Hinojosa, L. M., Gamboa-Dominguez, A., Hernandez-Gallegos, E., et al. (2011). Eag1 potassium channels as markers of cervical dysplasia. *Oncol. Rep.* 26, 1377–1383.
- Ouadid-Ahidouch, H., and Ahidouch, A. (2008). K<sup>+</sup> channel expression in human breast cancer cells: involvement in cell cycle regulation and carcinogenesis. *J. Membr. Biol.*

- 221, 1–6. doi: 10.1007/s00232-007-9080-6
- Ouadid-Ahidouch, H., Chaussade, F., Roudbaraki, M., Slomianny, C., Dewailly, E., Delcourt, P., et al. (2000). KV1.1 K(+) channels identification in human breast carcinoma cells: involvement in cell proliferation. *Biochem. Biophys. Res. Commun.* 278, 272–277. doi: 10.1006/bbrc.2000.3790
- Ouadid-Ahidouch, H., Le Bourhis, X., Roudbaraki, M., Toillon, R. A., Delcourt, P., and Prevarskaya, N. (2001). Changes in the K+ current-density of MCF-7 cells during progression through the cell cycle: possible involvement of a hetero-a-gogo K+ channel. *Receptors Channels* 7, 345–356.
- Ousingsawat, J., Spitzner, M., Puntheeranurak, S., Terracciano, L., Tornillo, L., Bubendorf, L., et al. (2007). Expression of voltage-gated potassium channels in human and mouse colonic carcinoma. *Clin. Cancer Res.* 13, 824–831. doi: 10.1158/1078-0432.CCR-06-1940
- Pardo, L. A., Contreras-Jurado, C., Zientkowska, M., Alves, F., and Stuhmer, W. (2005). Role of voltage-gated potassium channels in cancer. *J. Membr. Biol.* 205, 115–124. doi: 10.1007/s00232-005-0776-1
- Pardo, L. A., Del Camino, D., Sanchez, A., Alves, F., Bruggemann, A., Beckh, S., et al. (1999). Oncogenic potential of EAG K(+) channels. *EMBO J.* 18, 5540–5547. doi: 10.1093/emboj/18.20.5540
- Park, J. H., Park, S. J., Chung, M. K., Jung, K. H., Choi, M. R., Kim, Y., et al. (2010). High expression of large-conductance Ca2+-activated K+ channel in the CD133+ subpopulation of SH-SY5Y neuroblastoma cells. *Biochem. Biophys. Res. Commun.* 396, 637–642. doi: 10.1016/j.bbrc.2010.04.142
- Pettit, E. J., and Fay, F. S. (1998). Cytosolic free calcium and the cytoskeleton in the control of leukocyte chemotaxis. *Physiol. Rev.* 78, 949–967.
- Plummer, H. K. 3rd., Dhar, M. S., Cekanova, M., and Schuller, H. M. (2005). Expression of G-protein inwardly rectifying potassium channels (GIRKs) in lung cancer cell lines. *BMC Cancer* 5:104. doi: 10.1186/1471-2407-5-104
- Plummer, H. K. 3rd., Yu, Q., Cakir, Y., and Schuller, H. M. (2004). Expression of inwardly rectifying potassium channels (GIRKs) and beta-adrenergic regulation of breast cancer cell lines. *BMC Cancer* 4:93. doi: 10.1186/1471-2407-4-93
- Potter, M., Joulin, V., Roger, S., Besson, P., Jourdan, M. L., Leguennec, J. Y., et al. (2006). Identification of SK3 channel as a new mediator of breast cancer cell migration. *Mol. Cancer Ther.* 5, 2946–2953. doi: 10.1158/1535-7163.MCT-06-0194
- Prevarskaya, N., Skryma, R., and Shuba, Y. (2010). Ion channels and the hallmarks of cancer. *Trends Mol. Med.* 16, 107–121. doi: 10.1016/j.molmed.2010.01.005
- Price, M., Lee, S. C., and Deutsch, C. (1989). Charybdotoxin inhibits proliferation and interleukin 2 production in human peripheral blood lymphocytes. *Proc. Natl. Acad. Sci. U.S.A.* 86, 10171–10175. doi: 10.1073/pnas.86.24.10171
- Redmann, K., Muller, V., Tanneberger, S., and Kalkoff, W. (1972). The membrane potential of primary ovarian tumor cells *in vitro* and its dependence on the cell cycle. *Acta Biol. Med. Ger.* 28, 853–856.
- Ridley, A. J., Schwartz, M. A., Burridge, K., Firtel, R. A., Ginsberg, M. H., Borisy, G., et al. (2003). Cell migration: integrating signals from front to back. *Science* 302, 1704–1709. doi: 10.1126/science.1092053
- Rodriguez-Rasgado, J. A., Acuna-Macias, I., and Camacho, J. (2012). Eag1 channels as potential cancer biomarkers. *Sensors (Basel)* 12, 5986–5995. doi: 10.3390/s120505986
- Roger, S., Besson, P., and Le Guennec, J. Y. (2003). Involvement of a novel fast inward sodium current in the invasion capacity of a breast cancer cell line. *Biochim. Biophys. Acta* 1616, 107–111. doi: 10.1016/j.bbamem.2003.07.001
- Rouzaire-Dubois, B., Milandri, J. B., Bostel, S., and Dubois, J. M. (2000). Control of cell proliferation by cell volume alterations in rat C6 glioma cells. *Pflugers Arch.* 440, 881–888. doi: 10.1007/s004240000371
- Sachs, H. G., Stambrook, P. J., and Ebert, J. D. (1974). Changes in membrane potential during the cell cycle. *Exp. Cell Res.* 83, 362–366. doi: 10.1016/0014-4827(74)90350-4
- Santella, L., Ercolano, E., and Nusco, G. A. (2005). The cell cycle: a new entry in the field of Ca2+ signaling. *Cell. Mol. Life Sci.* 62, 2405–2413. doi: 10.1007/s00018-005-5083-6
- Sato, M., Suzuki, K., Yamazaki, H., and Nakanishi, S. (2005). A pivotal role of calcineurin signaling in development and maturation of postnatal cerebellar granule cells. *Proc. Natl. Acad. Sci. U.S.A.* 102, 5874–5879. doi: 10.1073/pnas.0501972102
- Schwab, A., Fabian, A., Hanley, P. J., and Stock, C. (2012). Role of ion channels and transporters in cell migration. *Physiol. Rev.* 92, 1865–1913. doi: 10.1152/physrev.00018.2011
- Schwab, A., Nechyporuk-Zloy, V., Fabian, A., and Stock, C. (2007). Cells move when ions and water flow. *Pflugers Arch.* 453, 421–432. doi: 10.1007/s00424-006-0138-6
- Schwab, A., Wulf, A., Schulz, C., Kessler, W., Nechyporuk-Zloy, V., Romer, M., et al. (2006). Subcellular distribution of calcium-sensitive potassium channels (IK1) in migrating cells. *J. Cell. Physiol.* 206, 86–94. doi: 10.1002/jcp.20434
- Smith, N. R., Sparks, R. L., Pool, T. B., and Cameron, I. L. (1978). Differences in the intracellular concentration of elements in normal and cancerous liver cells as determined by X-ray microanalysis. *Cancer Res.* 38, 1952–1959.
- Smith, P. L., Baukowitz, T., and Yellen, G. (1996). The inward rectification mechanism of the HERG cardiac potassium channel. *Nature* 379, 833–836. doi: 10.1038/379833a0
- Sontheimer, H. (2008). An unexpected role for ion channels in brain tumor metastasis. *Exp. Biol. Med.* 233, 779–791. doi: 10.3181/0711-MR-308
- Soroceanu, L., Manning, T. J. Jr., and Sontheimer, H. (1999). Modulation of glioma cell migration and invasion using Cl(-) and K(+) ion channel blockers. *J. Neurosci.* 19, 5942–5954.
- Sparks, R. L., Pool, T. B., Smith, N. K., and Cameron, I. L. (1983). Effects of amiloride on tumor growth and intracellular element content of tumor cells *in vivo*. *Cancer Res.* 43, 73–77.
- Stevenson, D., Binggeli, R., Weinstein, R. C., Keck, J. G., Lai, M. C., and Tong, M. J. (1989). Relationship between cell membrane potential and natural killer cell cytotoxicity in human hepatocellular carcinoma cells. *Cancer Res.* 49, 4842–4845.
- Strobl, J. S., Wonderlin, W. F., and Flynn, D. C. (1995). Mitogenic signal transduction in human breast cancer cells. *Gen. Pharmacol.* 26, 1643–1649. doi: 10.1016/0306-3623(95)00062-3
- Stuhmer, W., Alves, F., Hartung, F., Zientkowska, M., and Pardo, L. A. (2006). Potassium channels as tumour markers. *FEBS Lett.* 580, 2850–2852. doi: 10.1016/j.febslet.2006.03.062
- Stuhmer, W., and Pardo, L. A. (2010). K(+) channels as therapeutic targets in oncology. *Future Med. Chem.* 2, 745–755. doi: 10.4155/fmc.10.24
- Sundelacruz, S., Levin, M., and Kaplan, D. L. (2008). Membrane potential controls adipogenic and osteogenic differentiation of mesenchymal stem cells. *PLoS ONE* 3:e3737. doi: 10.1371/journal.pone.0003737
- Sundelacruz, S., Levin, M., and Kaplan, D. L. (2009). Role of membrane potential in the regulation of cell proliferation and differentiation. *Stem Cell Rev.* 5, 231–246. doi: 10.1007/s12015-009-9080-2
- Sundelacruz, S., Levin, M., and Kaplan, D. L. P. (2013). Depolarization alters phenotype, maintains plasticity of pre-differentiated mesenchymal stem cells. *Tissue Eng. Part A* doi: 10.1089/ten.tea.2012.0425.rev. [Epub ahead of print].
- Szaszi, K., Sirokmany, G., Di Ciano-Oliveira, C., Rotstein, O. D., and Kapus, A. (2005). Depolarization induces Rho-Rho kinase-mediated myosin light chain phosphorylation in kidney tubular cells. *Am. J. Physiol. Cell Physiol.* 289, C673–C685. doi: 10.1152/ajpcell.00481.2004
- Takanami, I., Inoue, Y., and Gika, M. (2004). G-protein inwardly rectifying potassium channel 1 (GIRK1) gene expression correlates with tumor progression in non-small cell lung cancer. *BMC Cancer* 4:79. doi: 10.1186/1471-2407-4-79
- Tokuoka, S., and Morioka, H. (1957). The membrane potential of the human cancer and related cells. I. *Gan* 48, 353–354.
- Trudeau, M. C., Warmke, J. W., Ganetzky, B., and Robertson, G. A. (1995). HERG, a human inward rectifier in the voltage-gated potassium channel family. *Science* 269, 92–95. doi: 10.1126/science.7604285
- Voloshyna, I., Besana, A., Castillo, M., Matos, T., Weinstein, I. B., Mansukhani, M., et al. (2008). TREK-1 is a novel molecular target in prostate cancer. *Cancer Res.* 68, 1197–1203. doi: 10.1158/0008-5472.CAN-07-5163
- Wagner, V., Stadelmeyer, E., Riederer, M., Regitnig, P., Gorishek, A., Devaney, T., et al. (2010). Cloning and characterization of GIRK1 variants resulting from alternative RNA editing of the KCNJ3 gene transcript in a human breast cancer cell line. *J. Cell. Biochem.* 110, 598–608. doi: 10.1002/jcb.22564
- Wang, H., Zhang, Y., Cao, L., Han, H., Wang, J., Yang, B., et al. (2002). HERG K+ channel, a regulator of tumor cell apoptosis and proliferation. *Cancer Res.* 62, 4843–4848.
- Wang, Y. F., Jia, H., Walker, A. M., and Cukierman, S. (1992).

- K-current mediation of prolactin-induced proliferation of malignant (Nb2) lymphocytes. *J. Cell. Physiol.* 152, 185–189. doi: 10.1002/jcp.1041520123
- Wang, Z. (2004). Roles of K<sup>+</sup> channels in regulating tumour cell proliferation and apoptosis. *Pflugers Arch.* 448, 274–286. doi: 10.1007/s00424-004-1258-5
- Weaver, A. K., Liu, X., and Sontheimer, H. (2004). Role for calcium-activated potassium channels (BK) in growth control of human malignant glioma cells. *J. Neurosci. Res.* 78, 224–234. doi: 10.1002/jnr.20240
- Wei, C., Wang, X., Chen, M., Ouyang, K., Song, L. S., and Cheng, H. (2009). Calcium flickers steer cell migration. *Nature* 457, 901–905. doi: 10.1038/nature07577
- Wicha, M. S., Liu, S., and Dontu, G. (2006). Cancer stem cells: an old idea—a paradigm shift. *Cancer Res.* 66, 1883–1890. discussion: 1895–1886. doi: 10.1158/0008-5472.CAN-05-3153
- Wilson, G. F., and Chiu, S. Y. (1993). Mitogenic factors regulate ion channels in Schwann cells cultured from newborn rat sciatic nerve. *J. Physiol.* 470, 501–520.
- Wonderlin, W. F., and Strobl, J. S. (1996). Potassium channels, proliferation and G1 progression. *J. Membr. Biol.* 154, 91–107. doi: 10.1007/s002329900135
- Wonderlin, W. F., Woodfork, K. A., and Strobl, J. S. (1995). Changes in membrane potential during the progression of MCF-7 human mammary tumor cells through the cell cycle. *J. Cell. Physiol.* 165, 177–185. doi: 10.1002/jcp.1041650121
- Woodfork, K. A., Wonderlin, W. F., Peterson, V. A., and Strobl, J. S. (1995). Inhibition of ATP-sensitive potassium channels causes reversible cell-cycle arrest of human breast cancer cells in tissue culture. *J. Cell. Physiol.* 162, 163–171. doi: 10.1002/jcp.1041620202
- Woodrough, R. E., Canti, G., and Watson, B. W. (1975). Electrical potential difference between basal cell carcinoma, benign inflammatory lesions and normal tissue. *Br. J. Dermatol.* 92, 1–7. doi: 10.1111/j.1365-2133.1975.tb03026.x
- Yang, M., Kozminski, D. J., Wold, L. A., Modak, R., Calhoun, J. D., Isom, L. L., et al. (2012). Therapeutic potential for phenytoin: targeting Na(v)1.5 sodium channels to reduce migration and invasion in metastatic breast cancer. *Breast Cancer Res. Treat.* 134, 603–615. doi: 10.1007/s10549-012-2102-9
- Zheng, Y. J., Furukawa, T., Ogura, T., Tajimi, K., and Inagaki, N. (2002). M phase-specific expression and phosphorylation-dependent ubiquitination of the ClC-2 channel. *J. Biol. Chem.* 277, 32268–32273. doi: 10.1074/jbc.M202105200
- Ziechner, U., Schonherr, R., Born, A. K., Gavrilova-Ruch, O., Glaser, R. W., Malesevic, M., et al. (2006). Inhibition of human ether a go-go potassium channels by Ca<sup>2+</sup>/calmodulin binding to the cytosolic N- and C-termini. *FEBS J.* 273, 1074–1086. doi: 10.1111/j.1742-4658.2006.05134.x
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