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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 Na^+, K^+, Ca^{2+}, and 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):

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

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 Na^+ channels (VGSCs), voltage-gated K^+ channels (K_v), and the Na^+/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 Na^+ level is markedly higher in tumors compared to non-cancerous tissues, whereas the 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 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 is 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.

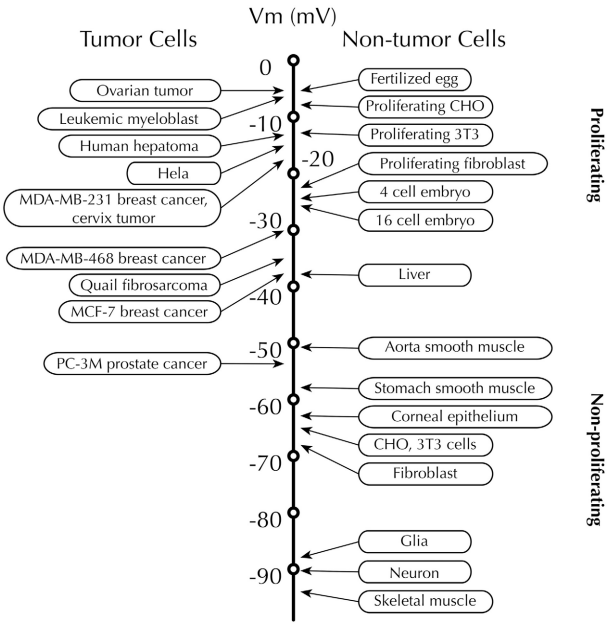

FIGURE 1 | Membrane potential (V_m) scale. Rapidly proliferating cancer cells possess depolarized V_m, while the V_m of quiescent cells is generally more negative. Proliferative somatic cells are also depolarized, suggesting that V_m is functionally instructive in cell development (Levin, 2007b). Scale adapted from Binggeli and Weinstein (1986), with additional data from Fraser et al. (2005); Mycielska et al. (2005); Yang et al. (2012).

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 Na^+/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 Cl^- channels and applying the activator ivermectin. Depolarization (caused by lowering the Cl^- concentration in the extracellular medium, which caused 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 Cl^- concentration, as predicted by Goldman–Hodgkin–Katz equation (Lobikin et al., 2012). Therefore, the depolarized V_m frequently found in

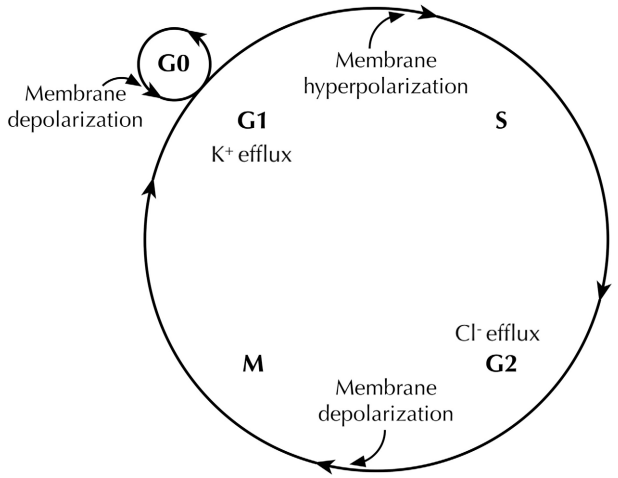

FIGURE 2 | Membrane potential (V_m) changes during the cell cycle. V_m undergoes hyperpolarization at G_1/S border, by virtue of K^+ efflux through various K^+ channels. Before cells enter M phase, increased Cl^- efflux accompanies V_m depolarization. Quiescent cells at G_0 stage show mitotic activities after V_m depolarization (Cone and Cone, 1976).

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_1/S checkpoint is generally required for S phase initiation. For example, depolarizing the cell membrane halts G_1/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_2/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_1 phase have a V_m of -9 mV and hyperpolarize to ~ -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^+ 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^+ efflux before entering the G_2 phase, a relatively high level of K^+ efflux during the M phase (Mills and Tupper, 1976; Boonstra et al., 1981). Given the diversity of K^+ 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^+ 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_v 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^+ 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_{EAG} is reduced to less than 5% in G_1 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_1 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_1 before the cells enter the S phase (Ouadid-Ahidouch et al., 2001). Interestingly, the hyperpolarization is accompanied by increased Ca^{2+}-activated K^+ (K_{Ca}) channel currents (Ouadid-Ahidouch et al., 2001), which might result from the elevated intracellular Ca^{2+} due to the increased electrochemical gradient (**Figure 3**) (Nilius and Wohlrab, 1992; Ouadid-Ahidouch and Ahidouch, 2008).

When K_{Ca} 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_{Ca} 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 $CD133^+$ subpopulation of SH-SY5Y cells (Park et al., 2010). Inhibiting K_{Ca} channels with iberiotoxin arrests D54-MG glioma cells in S phase, and leads to apoptosis (Weaver et al., 2004).

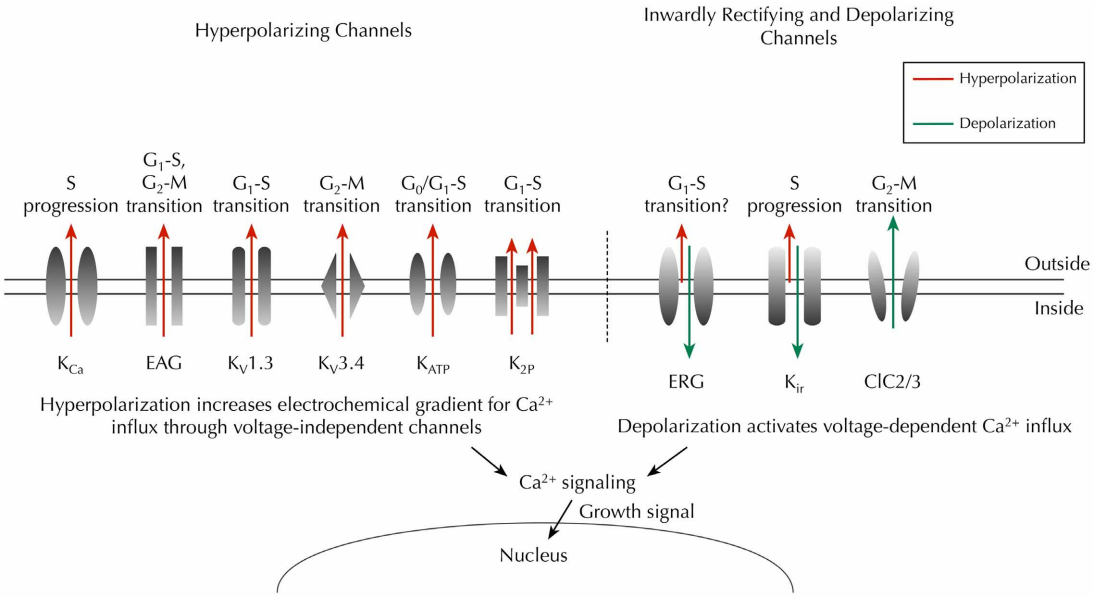

FIGURE 3 | Key ion channels that regulate V_m and cell cycle progression in cancer. Hyperpolarizing channels (outward I_K, red) would increase the driving force for Ca^{2+} influx through voltage-independent channels, whereas inwardly rectifying K^+ channels (predominantly inward I_K, green) and chloride channels (outward Cl^-, green) would depolarize the V_m, thus enabling activation of voltage-dependent Ca^{2+} influx (Schwab et al., 2012). Time- and domain-dependent Ca^{2+} signaling is then proposed to activate pathways that promote cell cycle progression and proliferation. Abbreviations: K_{Ca}, Ca^{2+}-activated K^+ channel; EAG, *ether à go-go* channel; K_V, voltage-gated K^+ channel; K_{ATP}, ATP-sensitive K^+ channel; K_{2P}, two-pore domain K^+ channel; ERG, EAG-related gene K^+ channel; K_{ir}, classic inward-rectifier K^+ channel; ClC2/3, chloride 2/3 channel.

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 $K_{ir}2.2$ (Lee et al., 2010), $K_{ir}3.1$, and $K_{ir}3.4$ (Plummer et al., 2004; Takanami et al., 2004; Plummer et al., 2005; Wagner et al., 2010). In contrast, overexpression $K_{ir}4.1$ 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-α-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).

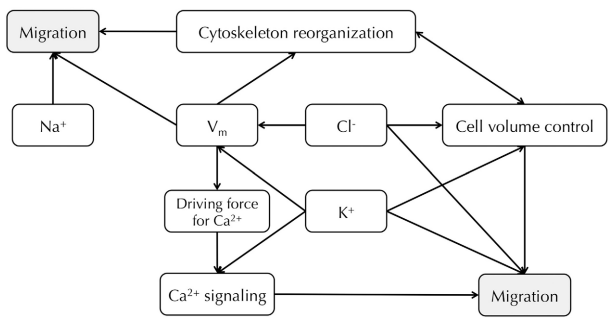

FIGURE 4 | Relationship between Na^+, K^+, Cl^- channels and V_m in cancer cell migration. V_m provides the driving force for Ca^{2+}, and downstream Ca^{2+} signaling leads to cell migration (Schwab et al., 2012). V_m also regulates cytoskeleton reorganization (Chifflet et al., 2003, 2004). Cl^- and K^+ channels both contribute to V_m regulation and cell volume control (Soroceanu et al., 1999; Sontheimer, 2008; Habela et al., 2009; Schwab et al., 2012). Inhibiting particular Na^+, K^+, and Cl^- channels can reduce cancer cell migration (Sontheimer, 2008; Brackenbury, 2012; 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, $K_{Ca}2.3$, 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, $K_{Ca}3.1$ 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

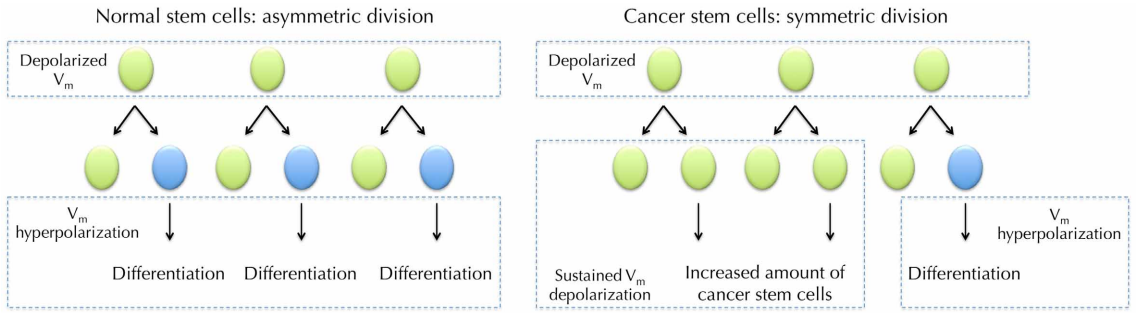

FIGURE 5 | V_m in normal stem cell (SC) differentiation and hypothesized role for V_m in cancer stem cells (CSCs). Depolarized V_m is needed during the maintenance of SCs. SC undergoes asymmetric division where it produces one copy of itself and one progeny that later differentiate into mature cells. The maturation requires V_m hyperpolarization (Sundelacruz et al., 2008). However, CSCs frequently undergo symmetric division, in which one CSC divides into two identical CSC progenies (Wicha et al., 2006). Sustained V_m depolarization may help to maintain the increasing CSCs in an undifferentiated state. Proliferation of CSCs then increases cancer malignancy.

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 varies ion channels/transporters may provide a convenient clinical approach.

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