Harnessing the membrane potential to combat cancer progression

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Abstract

Rapid fluctuations in the plasma membrane potential (V_m) provide the basis underlying the action potential waveform in electrically excitable cells; however, a growing body of literature shows that the V_m is also functionally instructive in non-excitable cells, including cancer cells. Various ion channels play a key role in setting and fine tuning the V_m in cancer and stromal cells within the tumor microenvironment, raising the possibility that the V_m could be targeted therapeutically using ion channel-modulating compounds. Emerging evidence points to the V_m as a viable therapeutic target, given its functional significance in regulating cell cycle progression, migration, invasion, immune infiltration and pH regulation. Several compounds are now undergoing clinical trials and there is increasing interest in therapeutic manipulation of the V_m via application of pulsed electric fields. The purpose of this article is to update the reader on the significant recent and ongoing progress to elucidate the functional significance of V_m regulation in tumors, to highlight key remaining questions and the prospect of future therapeutic targeting. In particular, we focus on key developments in understanding the functional consequences of V_m alteration on tumor development via the activation of small GTPase (K-Ras and Rac1) signalling, as well as the impact of V_m changes within the heterogeneous tumor microenvironment on immune cell function and cancer progression.

Introduction

The plasma membrane potential (V_m), the voltage difference between the cytosol and the extracellular environment, is determined by the unequal distribution and differential permeability of key ions including Na^+, K^+, Ca^{2+} and Cl^-. Rapid fluctuations in V_m provide the basis underlying the action potential waveform in electrically excitable cells; however, a growing body of literature shows that the V_m is also functionally instructive in non-excitable cells, including cancer cells. In addition to this, increasing evidence points to the V_m of various organelles, e.g. mitochondria[1], endosomes[2], playing key roles in regulating cellular behaviour, as well as likely interplay between plasma V_m and organellar V_m underlying unique signalling axes[3]. In our 2013 review article[4], we summarised current understanding in the field relating to V_m and its role in cancer progression. Since 2013, research in this area has progressed rapidly. The purpose of the present article is to update the reader on these key developments, with a focus on the role of the plasma V_m in cancer progression, and how it may present a unique therapeutic target.

V_m depolarization in cancer cells

Studies over a number of years have shown a remarkable correlation between V_m and malignancy, such that cancer cells have a more depolarized V_m compared to healthy normal cells (see[4] for references to individual studies dating back to 1959) (Figure 1). This correlation may, at least in part, be due to the fact that cancer cells are often highly proliferative, compared to terminally differentiated somatic cells, and thus V_m depolarization may be associated with a proliferative state. Indeed, V_m depolarization has been shown to initiate mitosis whereas hyperpolarization induced mitotic arrest[5], and artificial depolarization of *Xenopus laevis* embryos has been shown to promote malignant transformation, providing a sustaining proliferative signal[6]. Moreover, an exquisite rhythmic relationship exists between V_m and the cell cycle[7]. In addition to general depolarization seen in cancer cells

versus healthy normal counterparts, the V_m has been shown to fluctuate during cell cycle progression. V_m hyperpolarization has been shown to occur at the G_1/S checkpoint, remaining fairly hyperpolarized during the S phase, before becoming depolarised during the G_2/M transition.

A multiplicity of different ion channels has been shown to contribute to the fine tuning of V_m in cancer cells, thus promoting proliferation[4]. Of particular note is the involvement of various classes of K^+ channels, several of which serve not only as cancer biomarkers, but also as regulators of cell cycle progression and cellular proliferation, in part due to regulation of pro-proliferative signalling via altered intracellular Ca^{2+} [8]. These ion channel-dependent oscillations in V_m observed in cancer cells have led to the 'Celex hypothesis', which argues that K^+ channels are expressed on cancer cells relatively early on in tumor development, but as the disease progresses to a more invasive/metastatic phenotype, K^+ channel expression is downregulated and replaced by an upregulation of voltage-gated Na^+ channels (VGSCs)[9]. There is growing evidence in support of the notion that metastatic cancer cells are themselves electrically excitable and capable of firing action potentials[10–12]. In addition, the Celex hypothesis fits with the general depolarization of V_m observed in metastatic cancer cells versus non-metastatic cells at steady state[13]. Furthermore, the persistent inward Na^+ current carried by VGSCs expressed on metastatic cancer cells would be expected to contribute to this depolarization, and this, together with downregulated K^+ channel activity, promotes metastatic invasion[14].

Functional consequences of V_m alteration in tumor development

Despite the myriad different studies pointing to a link between V_m depolarization and cell proliferation, the mechanism(s) involved have, until relatively recently, remained elusive. In 2015, an elegant mechanism providing a direct link between V_m and mitogenic signaling was described for the first time[15]. In this model, V_m depolarization induced by the activities of

specific ion channels resulted in the redistribution of negatively charged phospholipids (phosphatidylserine and phosphatidylinositol 4,5-bisphosphate) within the inner leaflet of the phospholipid bilayer of the plasma membrane. This redistribution of phospholipids resulted in nanoclustering of the phosphatidylserine-anchored small guanosine triphosphate hydrolase (GTPase) K-Ras, leading to its activation and induction of the rapidly accelerated fibrosarcoma (RAF)–mitogen-activated protein kinase (MAPK) cascade, promoting proliferation. The authors thus delineated a mechanism by which K-Ras can function as a 'field effect transistor', linking the V_m to mitogenic intracellular signalling pathways[15].

A growing body of evidence implicates V_m in the regulation of other cellular processes that are integral to tumor development and the metastatic cascade, in addition to cancerous transformation and increased proliferation. For example, V_m depolarization may also promote apoptosis resistance: $K_v1.5$-dependent V_m hyperpolarization in cancer cell lines has been shown to inhibit voltage-gated Ca^{2+} channels, reducing intracellular Ca^{2+} levels and therefore inhibiting activation of nuclear factor of activated T-cells (NFAT), increasing apoptosis[3]. In addition, V_m fluctuations can also affect cancer cell migration; in part the mechanisms are likely dependent on V_m-mediated alteration of Ca^{2+} signalling, in turn leading to cytoskeletal reorganization[16,17]. V_m-dependent regulation of cancer cell migration may also be Ca^{2+}-independent: we recently showed that $Na_v1.5$-dependent V_m depolarization promotes redistribution of the phosphatidylserine-anchored small GTPase Rac1 at the leading edge of migrating metastatic breast cancer cells, resulting in Rac1 activation, cytoskeletal reorganisation and acquisition of a motile phenotype[18]. Thus, V_m-dependent activation of small GTPase signalling can not only increase mitogenic signalling[15], but can also promote migration in response to changes in the ionic tumor microenvironment (TME)[18].

V_m depolarization may also promote cancer cell invasion. Similar to depolarization-dependent migration, evidence suggests that persistent inward Na^+ current through VGSCs

enhances cellular invasive capacity[13]. Increased cytosolic Na^+ resulting from this persistent inward current has itself been shown to have a multiplicity of consequences on cancer cell behaviour[19], including altered pH buffering capacity, leading to activation of pH-dependent cysteine cathepsins and increased invasion[20]. An additional hitherto unexplored consequence of V_m depolarization is on the regulation of cancer stem cell differentiation. Various studies have shown that the V_m can regulate differentiation of human mesenchymal stem cells, with depolarization likely maintaining cells in an undifferentiated state[21]. It is not yet clear whether such a mechanism exists in the context of cancer stem cells; however, V_m depolarization may serve as a survival mechanism to preserve energy, as has been shown in immune cells, where high extracellular K^+ maintains T cell stemness[22].

Impact of V_m changes in the tumor microenvironment

Tumor progression is regulated by the crosstalk among components of the TME, which consists of tumor cells, immune cells, extracellular matrix (ECM) and stromal cells. Although the ionic composition of the TME is less well studied and therefore the functional roles of V_m in the various constituent TME cell types remains unclear, high local extracellular $[K^+]$ within mouse and human tumors has been shown to suppresses cluster of differentiation (CD) 8^+ T cell effector function in necrotic areas of the TME[23], highlighting the relevance of ionic reprogramming to tumor-infiltrating immune cells. This is supported by an *in vitro* study showing that high extracellular $[K^+]$ inhibits T cell proliferation, reduces pro-inflammatory cytokine production, including interferon (IFN)-γ and interleukin (IL)-2, and increases expression of the programmed death-1 (PD-1) receptor on T cells[24], leading to immunosuppression in PD-1 ligand (PD-L1)-expressing tumors.

Tumor ionic activity is closely related to T cell activation, differentiation, proliferation and apoptosis via Ca^{2+} signaling[25]. For example, K_{Ca} and K_V channels maintain a hyperpolarized V_m and increase the driving force for Ca^{2+} entry via the Ca^{2+} release-activated Ca^{2+} (CRAC)

channel Orai1, which in turn tunes T cell immunity[26]. Compared to quiescent T cells, stimulated T cells have distinct expression profiles of K_{Ca} and K_v channels, and blocking those channels impairs $CD4^+$ T cell proliferation and IL-2 secretion[27,28], and blockade of $K_{Ca}3.1$ channels inhibits the migration of $CD3^+$ T cells[29]. High availability of Na^+ in the TME[19] may increase the driving force for Na^+ entry into T cells via the Ca^{2+}-activated cation channel, transient receptor potential cation channel subfamily M (TRPM)4, whose activation leads to a depolarized phenotype and decreases cytosolic [Ca^{2+}], which may reduce IL-2 production and therefore dampen tumoricidal activity[30]. Furthermore, high extracellular [K^+], [Ca^{2+}] (both due to apoptosis/necrosis) and high [Na^+] in tumor hypoxic/necrotic regions, together with the abundance of H_2O_2, which activates TRPM2, can lead to Ca^{2+} influx-induced T cell apoptosis[31]. Together, these results raise the intriguing possibility that hyperpolarizing the V_m of cytotoxic effector cells may increase their efficiency in combating tumor cells.

For cancer cells to avoid immune eradication, there is usually a dysregulation in the interaction and balance between the effector immune cell and the regulatory immune cell population. Among the latter, tumor-associated macrophages (TAMs) are widely present within the TME, exhibiting a tumor-promoting (sometimes known as M2) phenotype. Blocking $K_{Ca}3.1$ channels on TAMs with the clotrimazole analogue TRAM-34 when co-cultured with colorectal cancer cells directed the TAMs towards an anti-inflammatory M2 phenotype[32]. Additionally, TRAM-34-treated mice exhibited a significant reduction in plasma pro-inflammatory cytokine levels, including IL-2, IL-6 and tumor necrosis factor-α (TNF-α), and increased the plasma levels of the anti-inflammatory cytokine IL-10[33]. Interestingly, TRAM-34 also increased IL-10 expression and secretion in immunosuppressive $CD4^+CD25^+$ regulatory T (T_{reg}) cells in an inflammatory bowel disease mouse model[34]. Future studies should investigate whether hyperpolarized V_m in immune cells leads to a reduction in immunosuppression in the TME.

Whether V_m itself can functionally regulate immune cell recruitment to the TME is not well understood. However, some evidence suggests that TMEs with an aberrant ionic composition may affect the chemotaxis of lymphocytes and other immune cell types. For example, migration of human lung mast cells towards various chemoattractants including C-X-C motif chemokine ligand 10 (CXCL10) is attenuated upon K_{Ca} channel blockade[35]. In addition, activating $K_{Ca}3.1$ channels on both $CD11c^{high}CD11b^{low}$ and $CD11c^{low}CD11b^{high}$ lung dendritic cells hyperpolarized their V_m, and blocking these channels with TRAM-34 impaired cysteine-cysteine chemokine ligand (CCL)19/CCL21-induced dendritic cell transmigration *in vitro*[36].

Future perspectives: therapeutic V_m targeting

The key role of various ion channels in setting and fine tuning the V_m in cancer and stromal cells within the TME (Figure 2) raises the possibility that the V_m could be targeted therapeutically using ion channel-modulating compounds. Indeed, a large body of preclinical research highlights a range of ion channel-modulating drugs as potential anti-cancer therapeutics, although the efficacy of many of these compounds is likely due to functional consequences in addition to V_m alteration[37]. Nonetheless, the emerging evidence highlighted in this Perspectives article points to the V_m as a viable therapeutic target, given its functional significance in regulating a range of intrinsic tumor features, including cell cycle progression, migration, invasion, immune infiltration and pH regulation (Figure 3). Excitingly, several of these ion transport-modulating interventions are now undergoing clinical trials[19,37]. Furthermore, there is increasing interest in therapeutic manipulation of cancer cell behaviour (and disruption of the V_m) via application of pulsed electric fields[38,39]. Clearly, for such interventions to be effective at targeting the V_m, the wider context of tissue-, organ-wide and long-range endogenous electric fields needs to be considered[40], as well as the possibility that cancer cells in certain tumor types may function as an electrical syncytium via gap-junctional communication[41]. A barrier to progression in this area remains the absence of

reliable methods to accurately quantify small V_m changes *in vitro* and *in vivo*, although encouraging progress is now being made in this regard[42,43]. In addition, further work is required to address whether the V_m-dependent effects reported thus far are generalisable across different tumor types. Finally, for such (drug-based or electrical) therapies to be effective, adequate selectivity for the lesion *vs.* healthy normal tissue is of paramount importance. Such selectivity may be achieved for electric field interventions by appropriate electrode replacement, however, challenges remain with respect to systemic drug application, when target ion channels may play a key role in regulating V_m in normal tissues, e.g. during mammary gland development/remodelling *vs.* cancer[18,44]. Further work is required to establish the effect(s) of local and systemic V_m-modulating therapies on both stromal cells in the TME (e.g. infiltrating immune cells) and surrounding normal cells. In conclusion, significant progress continues at pace to elucidate the functional significance of V_m regulation in tumors, and although key questions remain, there is the exciting prospect of its realization as a future therapeutic target.

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Authorship confirmation statement

WB had the original idea for this study. MY and WB wrote the manuscript. Both authors contributed to the interpretation and revising the manuscript. Both authors approved the final submitted version of the manuscript.

Authors' disclosure

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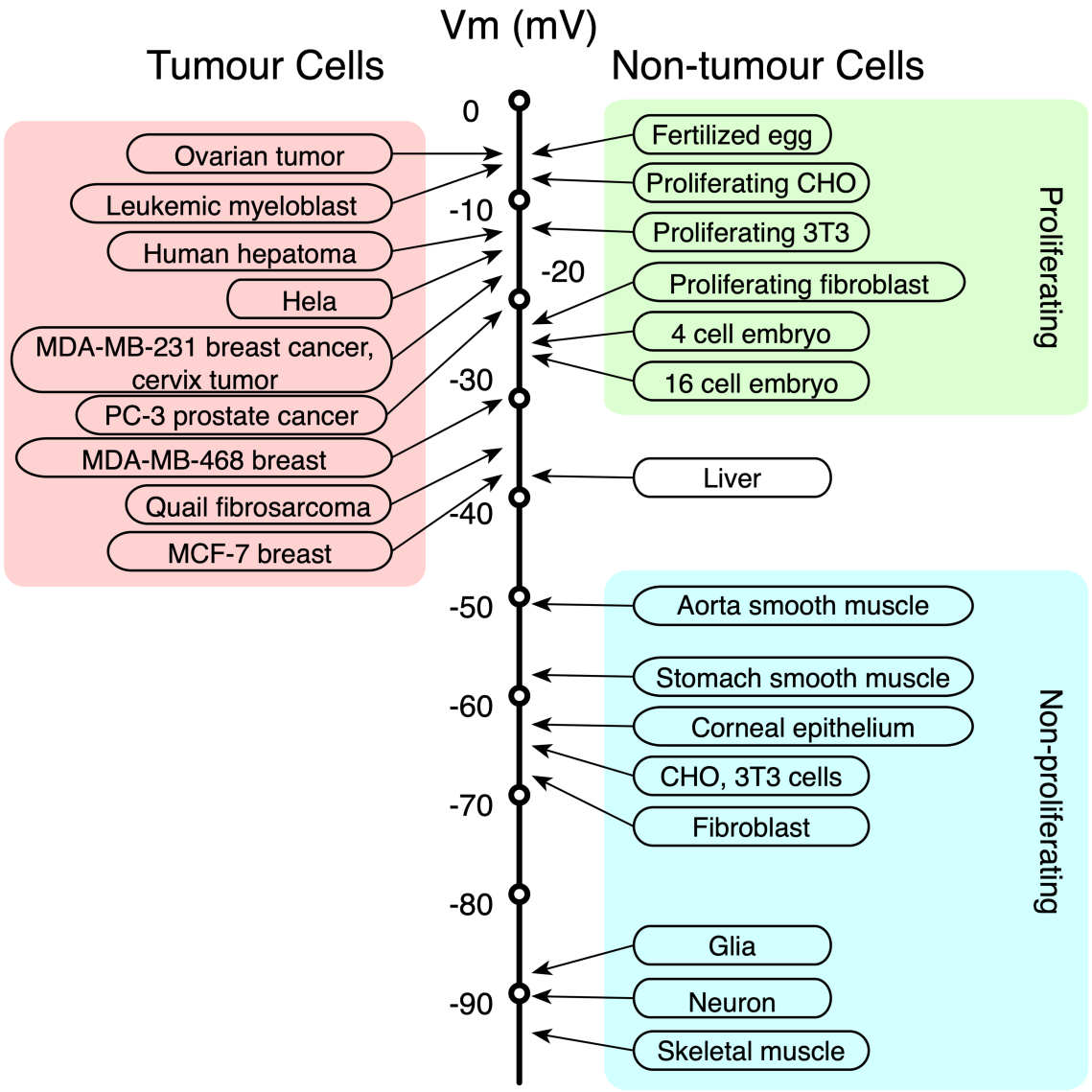

Figure 1. The membrane potential (V_m) scale across different cell types. Scale adapted from [4].

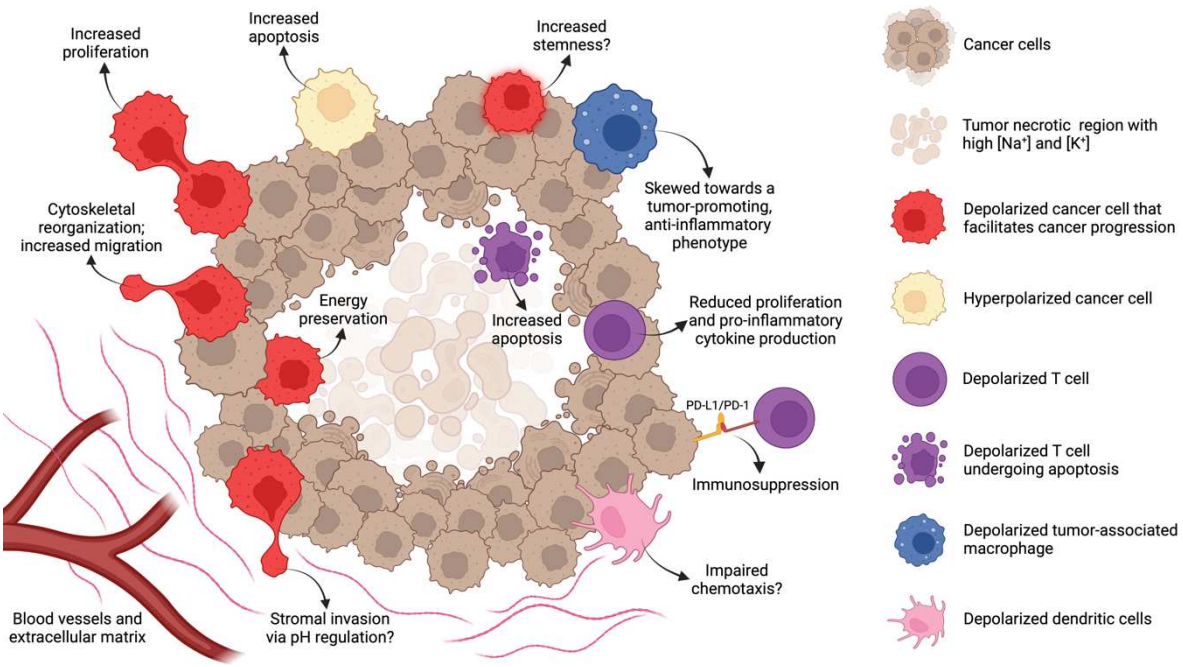

Figure 2. Altered membrane potential (V_m) in the tumor microenvironment (TME). Depolarized V_m in both cancer and immune cells in the TME can promote cancer progression by different mechanisms. PD-1: programmed cell death protein 1; PD-L1: programmed death-ligand 1. Figure created with BioRender.com.

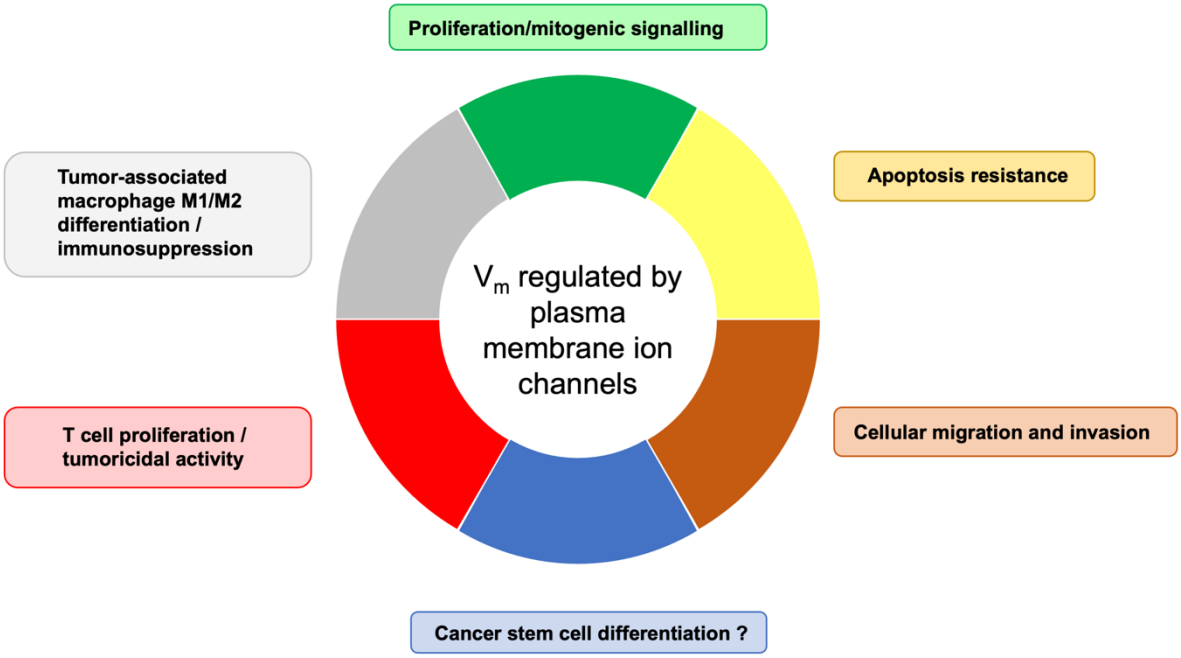

Figure 3. Summary of the key features of tumor development proposed to be regulated by the V_m.