Feature Review

Ion channels and the hallmarks of cancer

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Plasma membrane (PM) ion channels contribute to virtually all basic cellular processes and are also involved in the malignant phenotype of cancer cells. Here, we review the role of ion channels in cancer in the context of their involvement in the defined hallmarks of cancer: 1) self-sufficiency in growth signals, 2) insensitivity to antigrowth signals, 3) evasion of programmed cell death (apoptosis), 4) limitless replicative potential, 5) sustained angiogenesis and 6) tissue invasion and metastasis. Recent studies have indicated that the contribution of specific ion channels to these hallmarks varies for different types of cancer. Therefore, to determine the importance of ion channels as targets for cancer diagnosis and treatment their expression, function and regulation must be assessed for each cancer.

Introduction

Cancer is responsible for approximately 13% of deaths worldwide (http://www.who.int/mediacentre/factsheets/fs297/en/index.html). Pathogenesis involves dynamic changes in the genome that ultimately impair normal tissue homeostasis by driving the transformation of cells into malignant derivatives that exhibit uncontrolled multiplication and spreading. There are over 100 distinct types of cancer, which are defined by specific genotypes, and much research effort is directed towards identifying new genes with altered expressions in specific cancers. However, as defined by Hanahan and Weinberg [1], the vast catalog of cancer cell genotypes can be characterized in terms of six essential pathophysiological phenotypes that define malignant growth: 1) self-sufficiency in growth signals, 2) insensitivity to antigrowth signals, 3) evasion of programmed cell death (apoptosis), 4) limitless replicative potential, 5) sustained angiogenesis and 6) tissue invasion and metastasis.

Among the genes affected during malignant transformation, it is inevitable that those encoding PM ion channels are included. Ion fluxes through these channels define cell membrane potential and volume, are involved in intracellular signaling events and activate specific cellular responses. The latter two functions crucially depend on ion channels that transport Ca^{2+} (Box 1), the universal signaling ion. Thus, genetically determined alterations in the expression of different channels during cancer progression might, at least in part, be responsible for one or more of the pathophysiological features that define malignant growth.

The first important role ascribed to PM ion channels over 60 years ago was participation in cellular electrogenesis and electrical excitability. However, numerous subsequent studies have established the contribution of ion channels in virtually all basic cellular processes, including crucial roles in maintaining tissue homeostasis such as proliferation, differentiation and apoptosis. The concept of ion channel involvement in the abnormal progression of these processes emerged in the late 1980s with the realization that classical blockers of some channels, especially for K^+ and Ca^{2+} channels, are able to inhibit cellular proliferation or apoptosis *in vitro*. Much progress over the past two decades in the molecular identification of ion channel types and use of new research tools for the detection and manipulation of their expression have confirmed this concept, and have allowed further studies of other cancer-related processes such as malignant angiogenesis, migration and metastasis. The importance of such developments was highlighted in 2008 by the organization of the first international meeting of the scientists, oncologists and representatives of the pharmaceutical industry interested in understanding the role of ion channels in the development and progression of cancer and the possibility of their exploitation for cancer diagnosis and treatment [2].

A number of recent reviews have discussed the roles of ion channels in cancer from two principal standpoints: examining how specific ion channels are involved in certain cancer-related cellular behaviors such as proliferation, apoptosis, migration or angiogenesis [3–11], or examining the specific expression and functional profiles of various channels characteristic of certain human cancers [12–16]. In this review, we will discuss how the six pathophysiological hallmarks of cancer, as defined by Hanahan and Weinberg [1], depend on various types of ion channels, and evaluate their roles in metastasis and their potential as therapeutic and diagnostic targets.

Ion channels – biology and function

Ion channels are integral membrane proteins that allow the passive passage of certain ions into and out of the cell. The term "passive" refers to the fact that ions move

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through the channel only along their electrochemical gradient. Other than that ion channels are by no means passive structures because they are able to open and close (gate) in response to specific stimuli, selectively allow the passage of specific ion(s), sense various regulatory signals and react to physical and chemical characteristics of the surrounding medium.

There are many types of ion channels, which at the functional level are traditionally classified by either the stimulus that gates them or ion species that they pass. Molecular cloning has led to the identification of specific proteins within the families and subfamilies of homologous proteins that form homo- or multimeric channel complexes of specific functional type (Supplementary Table 1), enabling researchers to exactly pinpoint the channel involved in certain normal functions or malignant behaviors.

As they were discovered, ion channels were usually given abbreviated names that reflected either their specific functional features or origin. The same channel was sometimes named differently by various research groups involved in its discovery. This ultimately led to significant confusion in channel nomenclature. To avoid this, the International Union of Basic and Clinical Pharmacology (IUPHAR) committee on receptor nomenclature and drug classification (http://www.iuphar.org/nciuphar.html) adopted a unifying nomenclature, which together with the Human Genome Organization (HUGO) nomenclature committee (http://www.genenames.org/) provided the basis for modern channel classifications (Supplementary Table 1). In this review, we will cite the channel by its most popular name, often as it appeared in the original papers, but also provide its IUPHAR and HUGO designations at first mention. Alternative names for the channels involved in cancer hallmarks are also presented in Supplementary Table 2.

Ion channels in cancer hallmarks
In this section, we will describe the involvement of different types of ion channels in defining each of the six cancer hallmarks: 1) self-sufficiency in growth signals, 2) insensitivity to antigrowth signals, 3) evasion of programmed cell death (apoptosis), 4) limitless replicative potential, 5) sustained angiogenesis and 6) tissue invasion and metastasis.

Self-sufficiency in growth signals
Normal cells require mitogenic growth signals to switch from a quiescent to an active proliferative state. However, tumor cell proliferation is not solely dependent on external growth signals because these cells acquire significant growth autonomy. Three factors underlie the switch to the autonomic growth of tumor cells: 1) acquisition by a heterogeneous neoplastic cell population with the ability to produce and release intrinsic mitogens, which can act in an autocrine or paracrine manner; 2) alteration of the expression and/or properties of cell surface receptors and ion channels that accept growth signals and transmit them to downstream targets; and 3) deregulation of intracellular pathways that ultimately target gene expression.

Sources of growth signals
Although tumor cells are capable of secreting a number of mitogens, an additional powerful source of intrinsic growth

factors within tumors is represented by neuroendocrine (NE) differentiated cells. NE cells are normal components of many tissues; however, the abnormal expansion of this population from malignant precursor cells is a common characteristic of cancer progression. To various extents, NE differentiation, which usually indicates a poorer prognosis, appears in many human neoplasms including the most common carcinomas: colorectal [17], lung [18], breast [19] and prostate [20]. The primary function of malignant NE cells is the production of specific peptides and amine products that regulate the development of nearby cells in an endocrine/paracrine manner [21]. Neuropeptides found in tumors that can function as growth factors in cancer cells include parathyroid-hormone-related peptides, bombesin-related peptides, vasoactive intestinal peptides (VIPs), neurotensin, somatostatin, neuropeptide Y, serotonin and calcitonin [21].

Differentiation also requires Ca^{2+} signaling to progress. For instance, the NE differentiation of LNCaP (an androgen-dependent prostate cancer cell line) epithelial cells is associated with the increased expression of the $Ca_v3.2$ subtype of T type voltage-dependent Ca^{2+} channels [22], which have a specific "window current" (the current through voltage-gated ion channels flowing in the range of membrane potentials, in which channels are activated but not inactivated) that enables constant Ca^{2+} influx at around the resting membrane potential. These channels promote the formation of neuronal-like morphological features and facilitate the Ca^{2+}-dependent secretion of potential mitogenic factors, which in turn could drive the progression of disease towards an androgen-independent stage [23].

Higher levels of secretory activity of prostate cancer (PCa) cells might also result from the enhanced expression of the transient receptor potential (TRP, Box 1) channel family member TRPM8. TRPM8 is an ion channel better known as the cold receptor in sensory neurons that is responsive to cold and cooling compounds such as menthol, icilin and eucaliptol (reviewed in [24]). However, *TRPM8* was initially cloned as a prostate-specific gene whose expression increased during transformation to PCa [25]. In PCa, TRPM8 expression is mostly restricted to malignant epithelial cells that retain androgen dependence along with a fully differentiated luminal phenotype [26,27]. Because of its Ca^{2+} permeability, TRPM8 might promote the enhanced secretion of mitogens by these cells in response to factors that alter levels of phosphatidylinositol bisphosphate (PIP_2), lysophospholipids or polyunsaturated fatty acids, which are known to modulate channel activity [28–30]. A similar oncogenic role for TRPM8 might also be implicated in other common human cancers (breast, colon, skin) in which its expression is elevated [25].

In small cell lung carcinoma (SCLC) cells, which express phenotypic NE features, the release of the autocrine growth factor serotonin is largely controlled by the $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$ nAChR) [31]. The stimulation of this receptor by a high-affinity exogenous agonist, tobacco-specific carcinogen nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), activates the mitogenic Raf-1/MAPK/c-myc signaling pathway in SCLC cells because of the release and subsequent autocrine action of serotonin [31].
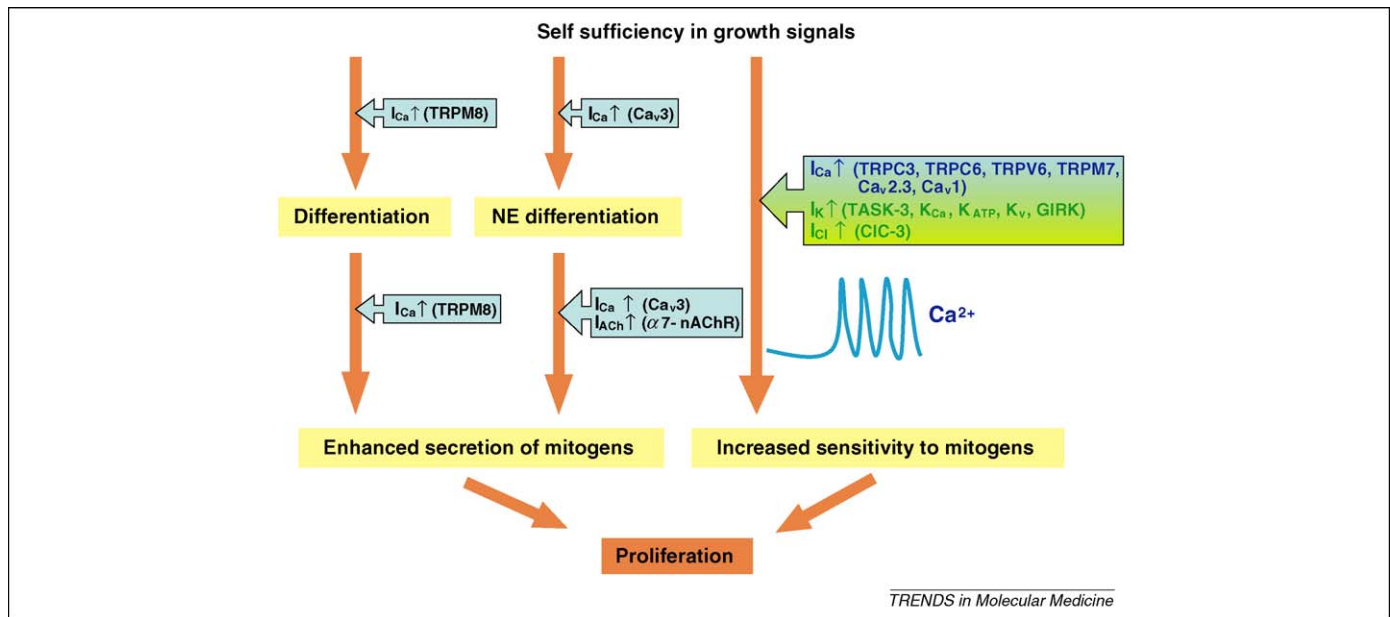
Figure 1. Self-sufficiency in growth signals. In this and other figures: block arrows indicate positive influence; upward or downward arrows near the designation of calcium (I_{Ca}), potassium (I_K) or chloride (I_{Cl}) membrane current indicate the up- or downregulation of the current, respectively; molecular designations of ion channels for which altered expression is responsible for the specified change in the current magnitude are presented in parenthesis after the currents. Tumor growth due to cell proliferation is determined by two factors: 1) the enhanced secretion of mitogenic factors by tumor cells and 2) the increased sensitivity of tumor cells to their action. The malignant differentiation of the apical secretory phenotype and NE phenotype, which is promoted by upregulated Ca^{2+} entry ($I_{Ca}\uparrow$) due to the overexpression of the TRPM8 cold receptor/channel and Ca_v3-type VGCCs, respectively, is in part responsible for the enhanced secretion of mitogenic factors, acting in an autocrine and paracrine manner. Ca^{2+}-permeable nAChR (Box 1) carrying ACh-activated inward current, I_{ACh}, may contribute to this process in some cancers. Ca^{2+} influx, primarily via TRPC3, TRPC6 and TRPV6 TRP members as well as Ca_v2.3 and Ca_v1-type VGCCs, participate in the generation of basal and mitogen-evoked periodic Ca^{2+} signals required for the activation of pro-proliferatory transcription factors. Enhanced potassium ($I_K\uparrow$) and chloride ($I_{Cl}\uparrow$) fluxes due to the overexpression of the TASK-3 K^+ channel and members of the K_{Ca}, K_{ATP}, K_v and GIRK K^+ channel families as well as the ClC-3 chloride channel enable the control of membrane potential and cell volume required for facilitated progression through the cell cycle.

Ca_v channels

In addition to the presence of excess mitogens, cancer cells also become hypersensitive to them by developing an intrinsic potential for uncontrolled proliferation because of the altered expression and/or function of the membrane receptors and ion channels that receive these external signals (Figure 1). It has long been understood that Ca^{2+} influx is required for cell-cycle progression and that a reduction in the levels of extracellular Ca^{2+} terminates the progression through the G1 phase (cell growth), causing cells to remain at the G1/S boundary. Consistent with this, all mitogens induce the Ca^{2+} entry pathway(s). Of the voltage-gated Ca^{2+} channels (Ca_v, Box 1), members of the low voltage-activated Ca_v3 subfamily, and in particular the window current-endowed Ca_v3.2 channels, are implicated in proliferation (reviewed in [6]). Ca_v3 channels are expressed in a number of cancer-derived cell lines, and their most specific blocking agent mibefradil shows antiproliferative action [6,32]. The increased expression of Ca_v3 channels is associated with the S phase (DNA synthesis) of the cell cycle, which is characterized by the highest levels of transcriptional activity. Their functional involvement at this point results from the hyperpolarization that accompanies the transition into the S phase [15] and drives channel recovery from inactivation. The siRNA-mediated silencing of Ca_v3.1 channels expressed in certain esophageal carcinoma cell lines can reduce cell proliferation via the p53 tumor-suppressing transcription factor-dependent pathway, leading to the upregulation of cell-cycle arrest protein p21 [32].

The expression of high voltage-activated Ca_v channels in non-excitable cells is limited, and even if these channels are present they require depolarization to become activated. However, these channels might also modulate proliferation either because of the more depolarized resting membrane potential (V_m) of cancer cells [33] or because the action of mitogens might involve depolarization. Indeed, Ca^{2+} influx through L-type channels has been implicated in the pro-proliferative action of a known mitogen endothelin-1 (ET-1) on the SPC-A1 human lung adenocarcinoma cell line [34]. Furthermore, the modulation of the growth ID8 tumorigenic mouse ovarian surface epithelial cell line via the follicle-stimulating hormone receptor (FSH-R3) has been shown to involve a signaling pathway that includes the Ca_v2.3 (R-type) channel [35].

TRP channels

The expression of a number of Ca^{2+} permeable TRP channels is altered in cancer [36]. Among these, the first to be identified were the epithelial Ca^{2+} transporters TRPV5 and TRPV6, cold/menthol receptor TRPM8, melanoma-specific TRPM1 (melastatin) and heat/capsaicin receptor TRPV1 [10,36]. The enhanced expression of the highly Ca^{2+}-selective TRPV6 channel has been detected in primary tumors of prostate, colon, breast, thyroid and ovary as well as in cell lines derived from these common human tumors (e.g. LNCaP and PC-3 prostate cancer, SW480 colorectal cancer and T47D breast cancer cell lines) [10,37–39]. TRPV6 mRNA expression levels have been shown to be 2–15-fold upregulated in breast cancer tissues compared with normal breast tissue, whereas TRPV6 regulation by estrogen, progesterone, tamoxifen and 1,25-dihydroxyvitamin D has been shown to affect breast cancer cell proliferation [39].

TRPV6 expression levels are significantly increased in high-grade prostate tumors with a Gleason score ≥ 7, making this a prognostic marker of PCa progression [40]. The siRNA-mediated silencing of TRPV6 expression slows down the proliferation of LNCaP human PCa cells, decreases their accumulation in the S phase of the cell cycle and lowers the expression of the proliferating cell nuclear antigen (PCNA) [41]. The pro-proliferative function of TRPV6 involves supporting Ca^{2+} entry, which is required for the activation of the Ca^{2+}/calmodulin/calcineurin-dependent transcription factor NFAT (nuclear factor of activated T cell) [41]; its transcriptional activity also alters the expression of cell-cycle regulators. It is notable that in primary human PCa epithelial cells, the same proliferation-promoting Ca^{2+}/calmodulin/calcineurin/NFAT pathway can be activated by Ca^{2+} entry through another TRP member TRPC6, whose activation is controlled by the α1-adrenoceptor and lipid messenger diacylglycerol (DAG) [42]. Thus, it seems that the TRPV6 channel is involved in the control of the basal proliferation of PCa cells, whereas TRPC6 might support the direct mitogenic effect of catecholamines via sympathetic nerves. Given that TRPV6 expression is strongly vitamin D-dependent [43], the vitamin D metabolism might also play an important role in these processes.

The pro-proliferative and/or antiapoptotic roles of the cold/menthol-sensitive TRPM8 channel are still poorly defined [10]. Because PCa cells might express two TRPM8 splice variants – the "classical" one localized in the PM and the truncated one localized in the membrane of endoplasmic reticulum (ER) [27] – it is possible that the role of TRPM8 might depend on its preferred localization, which in turn is a variable of PCa stage and androgen receptor (AR) status.

The TRPM1 channel has been identified to exhibit decreased expression during the transition of melanoma cells from the low metastatic to high metastatic phenotype [44]. Although its role in proliferation has been suggested, this aspect of TRPM1 function requires further investigation. Yet another member of the TRPM subfamily, redox state- and Mg^{2+}-sensitive TRPM7, was recently reported to be specifically overexpressed in large breast tumors [45], and its silencing in the MCF-7 breast cancer cell line reduced cell proliferation and basal intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), suggesting that TRPM7 is involved in the proliferative potential of breast cancer cells, most likely by regulating Ca^{2+} influx.

K^+ channels

K^+ channels are central to the maintenance of V_m and are, therefore, an integral part of all cells. Because many types of these channels allow the efflux of the dominant intracellular cation K^+, they are important for the osmotic regulation of cell volume, whilst by functioning in concert with the Na^+-H^+ exchanger and Na^+-K^+ ATPase they contribute to the regulation of intracellular pH (pH_i) [46]. Membrane potential, cell volume and pH_i all change during cell-cycle progression, making K^+ channels important determinants in physiological and pathological proliferation. K^+ channel activity is altered during progression through the cell cycle, and the inhibition of K^+ channels has

an antiproliferative effect. Hyperpolarization due to K^+ channel activation is required for the initiation of the G_1 phase of the cell cycle [47]. Usually, cancer cells have less negative V_m than normal cells [33]; therefore, it is likely that they would require a higher expression of certain types of K^+ channels to produce transient hyperpolarization in response to factors that change during cell-cycle progression (such as mitogens, ATP level, second messengers, $[Ca^{2+}]_i$, pH, osmolarity and membrane stretch).

The hyperpolarization of MCF-7 human breast cancer cells during their progression through G0/G1 and into S phase has been shown to depend on the ATP-sensitive K^+ channels K_{ATP} [48]. Their progression through the early G1 phase requires the activation of human ether-a-go-go-related gene (HERG, Erg1, K_v11.1) K^+ channels, whereas in G1 and at the G1/S checkpoint their membrane potential is primarily determined by intermediate conductance Ca^{2+}-dependent (IK_{Ca} or K_{Ca}3.1) K^+ channels [49]. Interestingly, K_{ATP} and G-protein-coupled inwardly rectifying K^+ channels (GIRK) are subject to upregulation by neuropeptide somatostatin [50], which can be released by NE cells. The overexpression of members of the GIRK K^+ channel subfamily has also been documented in native human breast carcinomas [51] and lung cancer cell lines [52]. The growth of breast cancer cell lines is dependent on the β-adrenoreceptor signaling pathway because of its functional coupling with the GIRK1 (K_{ir}3.1, *KCNJ3*) channels [53]. The β-adrenergic pathway is also implicated in the growth regulation of lung, pancreas and colon adenocarcinomas by the autonomic nervous system [54].

Oncogenic proliferation-promoting properties are also attributed to some members of the background 2P-domain (K_{2P}, "P" comes from "pore") K^+ channels that regulate cell V_m in response to physical and chemical factors such as membrane stretch, temperature, osmolarity, lipid messengers and external and internal pH [3]. Breast and lung cancers are characterized by an overexpression of K_{2P} member TASK-3 (K_{2P}9.1, *KCNK9*) [55], and cells with the heterologous overexpression of TASK-3 have been shown to acquire tumorigenic potential in experimental animal models. Yet another K_{2P} member TREK-1 (K_{2P}2.1, *KCNK2*) was also shown to play a pro-proliferative role in PCa cells [56].

In addition to voltage-independent K^+ channels, which operate close to V_m, voltage-gated channels (K_v) that require depolarization to open are implicated in proliferation. For instance, in T84 human colonic carcinoma cells only K_v channels, including the oncogenic Eag1 (K_v10.1, *KCNH1*) channel [4,57,58], have demonstrated pro-proliferative functions, despite these cells also expressing other K^+-channel types [59]. The Eag1 channel is normally present in the brain and at minor levels in peripheral tissues, but its expression is significantly increased in several human cancers [58,60]. The pharmacological, siRNA- or antibody-mediated inhibition of endogenous Eag1 commonly suppresses cell proliferation, whereas the heterologous overexpression of Eag1 enhances proliferation [57]. A known mitogen, insulin-like growth factor-1 (IGF-1), which stimulates cell proliferation either via the MAPK or phosphatidylinositol 3-kinase (PI3K) signaling pathways, has been shown to increase both the activity and
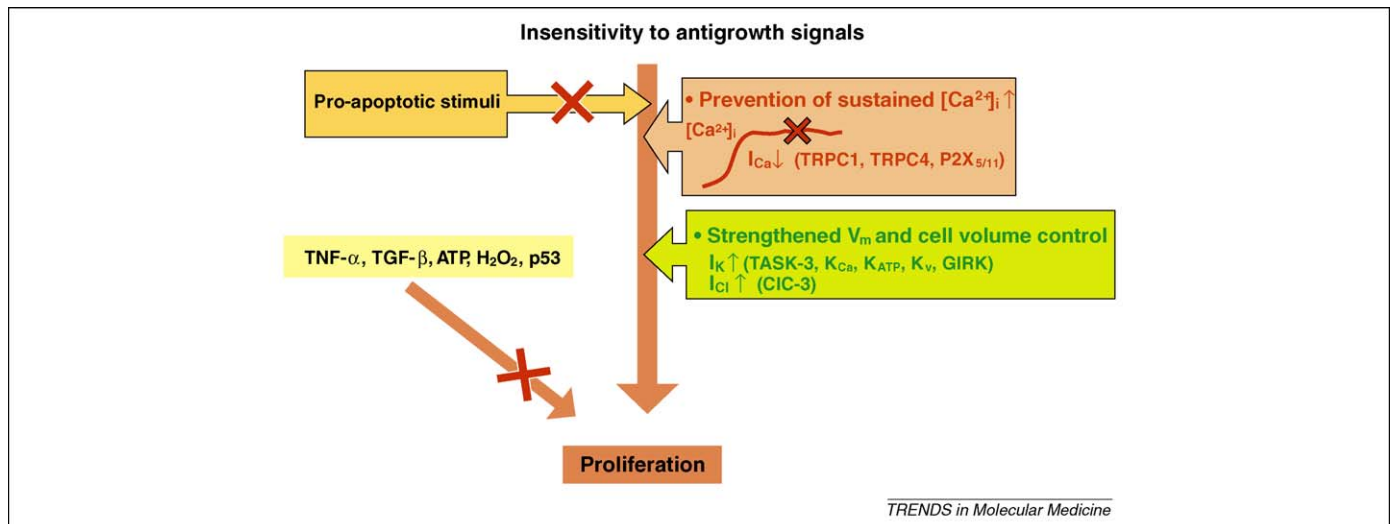
Figure 2. Insensitivity to antigrowth signals. In this and in other figures: block arrows crossed indicate that the respective positive influence is strongly impeded. The insensitivity of cancer cells to antigrowth signals that induce proliferation arrest, apoptosis or differentiation is determined by an expression pattern of membrane channels that prevents the sustained increase of cytosolic Ca^{2+} ($[Ca^{2+}]_i$) (i.e. the downregulation of I_{Ca} due to the underexpression of putative store-dependent TRP channel family members, TRPC1/TRPC4, and P2X receptors, $P2X_{5/11}$) and provide for strengthened membrane potential (V_m) and cell volume control (i.e. the upregulation of I_K and I_{Cl} due to the overexpression of the TASK-3 K^+ channel and members of the K_{Ca}, K_{ATP}, K_v and GIRK K^+ channel families as well as the ClC-3 chloride channel).

expression of Eag1 channels in MCF-7 human breast cancer cell lines [61], suggesting that the induction of Eag1 is essential in the mechanism of IGF-1 mitogenic action. However, the proliferative effects of K_v channels might result from indirect effects on intracellular Ca^{2+} signaling and pH_i, which require tight regulation during the cell cycle.

By allowing the effective redistribution of ions to compensate for osmolarity changes from any side of the membrane, K^+ and Cl^- channels represent important determinants of cell volume regulation [62]. The higher expression of K^+ channels promotes the tighter control of cell volume (Figure 1) within a relatively narrow window, which enables the maintenance of the optimal concentrations of cell-cycle regulatory proteins and solutes to ensure the highest proliferation rate [63].

Insensitivity to antigrowth signals
Tissue homeostasis is supported by the combined action of multiple mitogens and extracellular growth inhibitory signals that limit proliferative activity by inducing proliferation arrest, apoptosis or differentiation. The best described of these inhibitory signals are the cytokine tumor necrosis factor-alpha (TNF-α) and the secreted peptide transforming growth factor-beta (TGF-β). These both act via their respective surface receptors to activate a number of intracellular signaling pathways that eventually lead to gene expression. Among the important functional consequences of TNF-α and TGF-β action are proliferation arrest and apoptosis (reviewed in [64,65]), which positions them as major tumor suppressors. However, in human cancers their signaling activities are often modified, switching them from tumor suppressors at the early stages of cancer to tumor promoters at the late stages. Antigrowth effects are also associated with increased levels of extracellular ATP and some other nucleotides, which by acting via metabotropic Ca^{2+}-mobilizing purinergic P2Y receptor subtypes are capable of inducing growth arrest and/or

apoptosis in a number of common human malignancies, including prostate [42,66], esophageal [67], colon [68], ovarian [69] endometrial [70] and bladder [71] cancers.

Ca^{2+} entry channels
The insensitivity to antigrowth signals exhibited by malignant cells largely results from their perturbed Ca^{2+} homeostasis, which involves the altered expression of Ca^{2+} permeable ion channels (Figure 2). In general, the action of growth inhibitory signals involves a sustained decrease in the levels of ER Ca^{2+} stores along with an increase in $[Ca^{2+}]_i$ because of store-operated Ca^{2+} entry (SOCE). For example, these events have been shown to accompany the antigrowth effects of both TNF-α [72] and ATP [42,66] in PCa cells. In these cells, the ability of ATP to induce growth arrest was significantly compromised by the transient knockdown of store-dependent TRP channels TRPC1 and TRPC4 [42]. Furthermore, the heterologous overexpression of TRPC1 in the IEC-6 rat intestinal epithelial cell line was shown to enhance TNF-α-induced apoptosis with the simultaneous repression of the prosurvival and proproliferative branch of TNF-α-mediated signaling involving transcription factor NF-κB [73].

Thus, to become insensitive to growth inhibitory signals, cancer cells must develop protective mechanisms against reductions in ER Ca^{2+} store content and the subsequent activation of SOCE. Consistent with this, ATP-induced Ca^{2+} signaling via P2Y receptors in thyroid cancer cells showed specific impairment of the IP_3-linked Ca^{2+} release mechanism and activation of SOCE compared with normal human thyrocytes [74]. However, in the MCF-7 breast cancer cell line, ATP-activated $P2Y_2$ receptor-linked Ca^{2+} signaling induced a pro-proliferative response [75], highlighting the potential differences between cancer types. As shown by Thebault et al. [42], the functional consequence of Ca^{2+} entry might be determined by the spatial colocalization of the respective membrane channel(s) with the effector molecule(s) that transmits signals

to the downstream targets. Such colocalization might be specific for each type of cancer cell.

In human HT-1376 bladder carcinoma cells and hormone-refractory PCa cell lines (PC-3 and DU-145), the growth inhibitory action of ATP has been found to involve ionotropic $P2X_5$ and/or $P2Y_{11}$ purinergic receptors with the further recruitment of Ca^{2+}-independent apoptotic mechanisms [71,76]. The decreased expression or lack of these receptors in some cancers might, therefore, represent constraining factors for the antigrowth activity of ATP.

K^+ channels

Reduced levels of TNF-α-triggered apoptosis have been reported to accompany the heterologous overexpression of the oncogenic TASK-3 K^+ channel in C8 mouse fibroblasts [77]. However, it is more commonly observed that higher levels of K^+ channel expression and the associated enhancement of K^+ efflux promote apoptosis (see next section). Therefore, it is possible that either the indirect effects of TASK-3 on Ca^{2+} homeostasis via altered V_m or specific coupling between TASK-3 and TNF-α-mediated signaling might explain these findings. Such coupling might be suggested, particularly for the large conductance Ca^{2+}-dependent K^+ channel (BK_{Ca}) and another important growth inhibitory signal, the p53 tumor-suppressing transcription factor, which when activated by diverse carcinogens activates the transcription of multiple anticancer proteins and leads to growth arrest and/or apoptosis. The pharmacological blockade of BK_{Ca} channels in human HeLa cervical and A2780 ovarian cancer cell lines has been reported to induce apoptosis and cell-cycle arrest in the G1 phase, accompanied by the increased expression of p53 [78], suggesting that BK_{Ca} channel activity is required to keep p53 under negative control to prevent its antitumor actions.

The expression of the HERG (K_v11.1) K^+ channel, a close relative of the pro-proliferative Eag1 (K_v10.1) channel, has also been reported to interfere with the action of antigrowth signals. A number of cancer cell lines with an overexpression of HERG have firstly, exhibited markedly enhanced apoptosis in response to hydrogen peroxide (H_2O_2) or high concentrations of TNF-α (1–10 ng/ml) and secondly, facilitated proliferation in response to low concentrations of TNF-α (0.1–1 ng/ml) [79]. HERG-overexpressing cells also showed higher basal and TNF-α-stimulated activity of the nuclear transcription factor NF-κB. These data suggest that the ultimate effect of antigrowth signals on tumor progression might crucially depend not only on K^+ channel type, but also on the concentration of antigrowth signals.

Evasion of programmed cell death (apoptosis)

Apoptosis is integral to normal tissue homeostasis, and abnormalities in apoptotic functions underlie the pathogenesis of many diseases. In general, an excess of apoptosis can lead to tissue degeneration, whereas a deficiency can lead to cancer. The molecular machinery of apoptosis – from initiation to the final phagocytosis of cellular remnants – is complex, involving many molecular players and signaling pathways [80]. However, irrespective of whether it is part of a physiological or pathological process, it always involves Ca^{2+} influx followed by the recruitment of three major Ca^{2+}-dependent apoptotic mechanisms: mitochondrial, cytoplasmic and ER-mediated (reviewed in [81,82]).

Ca^{2+} homeostasis and Ca^{2+} entry channels

To effectively evade apoptosis, cancer cells must utilize mechanisms that substantially reduce or even prevent Ca^{2+} influx, for example by downregulating the expression of Ca^{2+} permeable channels and/or the signaling pathways that lead to their activation (Figure 3). Consistent with this, hormone-refractory apoptosis-resistant phenotypes of PCa cells are characterized by markedly reduced levels of SOCE [83–85], which prevents Ca^{2+} overload in response to pro-apoptotic stimuli, thereby reducing the effectiveness of mitochondrial and cytoplasmic apoptotic pathways. However, further studies are needed to identify the molecular components of store-operated channels (SOCs) that contribute to the reduction of SOCE.

Decreased levels of the expression of Ca^{2+} permeable channels with activation mechanisms other than store depletion also contribute to the ability of cancer cells to evade apoptosis. For instance, the antisense knockdown of TRPM2 (an endogenous ADP-ribose-sensitive, cyclic ADP-ribose-sensitive and H_2O_2-sensitive TRP member) in rat insulinoma RIN-5F cells and the U937 monocyte cell line has been shown to significantly suppress Ca^{2+} influx and cell death induced by H_2O_2 and TNF-α, whereas the heterologous overexpression of this channel enhanced H_2O_2-induced apoptosis [86]. Unexpectedly, the lack of the TRPV6 channel, rather than of the capsaicin receptor TRPV1, was found to suppress apoptosis of gastric cancer cells under capsaicin treatment [87].

Apart from the TRP channel family, ionotropic purinergic $P2X_7$ receptors (Box 1) play an important role in evading apoptosis of cervical cancer cells. In normal cervical epithelial cells, the activation of $P2X_7$ receptors induces apoptosis via the Ca^{2+}-dependent mitochondrial pathway [88]. However, in cervical cancer cells, decreased $P2X_7$ expression together with the dominant-negative action of a short $P2X_7$ splice variant results in the significant downregulation of the functional $P2X_7$ receptor, thereby preventing the Ca^{2+} influx required to trigger apoptosis [88].

K^+ channels

In addition to alterations in Ca^{2+} homeostasis, apoptosis is characterized by a series of changes that lead to V_m decay, cell shrinkage, DNA breakdown and finally phagocytosis. The diverse external and internal stimuli that trigger apoptosis have been shown to involve the loss of intracellular K^+ due to enhanced K^+ efflux, which is required for early apoptotic cell volume decrease (AVD) as well as for releasing inhibition by high K^+ levels of endogenous death-executing caspases and DNA-degrading endonucleases [89]. The efflux of K^+ to an extent that overrides the capacity of Na^+-K^+ ATPase to sustain the transmembrane K^+ gradient also causes the decay of V_m. Therefore, to evade apoptosis malignant cells must prevent the loss of
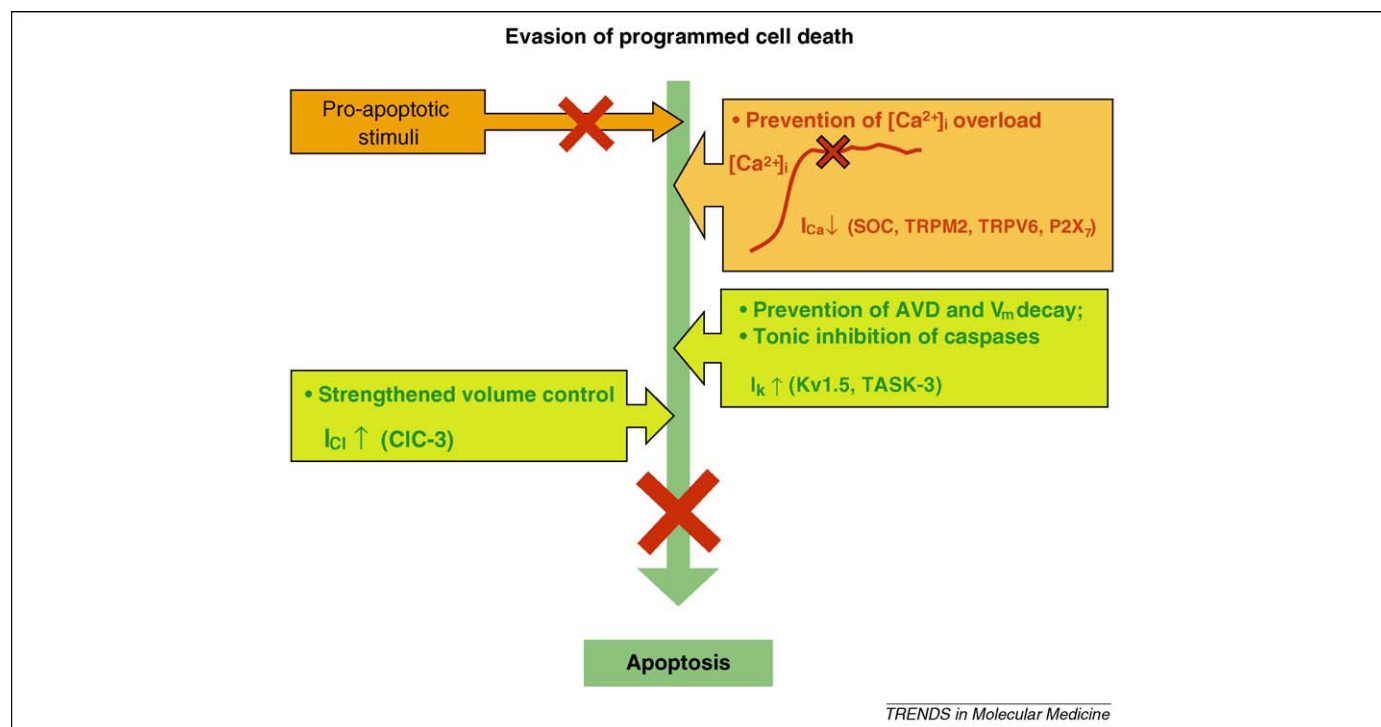
Figure 3. Evasion of programmed cell death (apoptosis). The enhanced apoptosis resistance of cancer cells involves: 1) the downregulation of I_{Ca} primarily due to the underexpression of SOCs, Ca^{2+} permeable TRP members TRPM2 and TRPV6 and $P2X_7$ purinergic receptor/channels, preventing sustained cytosolic Ca^{2+} overloads ($[Ca^{2+}]_i$); 2) the downregulation of I_k due to the underexpression of the TASK-3 K^+ channel and members of the K_v K^+ channel family, preventing the loss of intracellular K^+ and associated cell shrinkage (AVD) and the decay of membrane potential (V_m) as well as providing for the tonic inhibition of caspases; and 3) the upregulation of I_{Cl} due to the overexpression of the ClC-3 chloride channel, strengthening the cell volume control.

intracellular K^+ by downregulating PM K^+ channels (Figure 3). Consistent with this, several human cancers are characterized by higher mitochondrial membrane potential and a lower expression of the redox-sensitive K^+ channel $K_v1.5$, with both factors contributing to the enhanced resistance to apoptosis of cancer cells relative to normal cells [90]. The pharmacological blockade of $K_v1.5$ channels in SGC7901 gastric cancer cells has also been shown to enhance resistance to apoptosis-inducing chemotherapeutic drugs (adriamycin, cisplatin, vincristine and 5-fluorouracil) [91]. In addition, the lower activity of the TASK-3 channel has been correlated with the increased survival of glioma cells [92]. However, when assessing the effects of K^+ channels it is important to consider their indirect effects on the Ca^{2+} dependence of apoptosis occurring through the control of cell V_m and the associated Ca^{2+} influx. For instance, this mechanism was implicated in the apoptosis of HepG2 human hepatoblastoma cells induced by the K^+ channel blocker 4-AP [93]. In addition, the involvement in the antiapoptotic effects of the expression of oncogenic TASK-3 channel [77] mentioned above cannot be excluded.

Cl⁻ channels
The transition of cancer cells to apoptosis-resistant phenotypes is associated with an increased capability for regulatory volume decrease (RVD; the restoration of cell volume in response to hypoosmotic stress) because of the enhanced expression of volume-regulated anion channels (VRACs). The latter, at least in some cell types, can involve ClC-3 [13,94,95] (Figure 3), a member of the

ClC family, which is mostly known to function as endosomal Cl^-/H^+ exchanger, but can also function as PM Cl^- channel (Supplementary Table 1). Consistent with this, the overexpression of ClC-3 in human bronchial epithelial cells (HBECs) has been reported to inhibit TGF-β-induced apoptosis [96]. Owing to its preferential targeting to intracellular compartments and Cl^-/H^+ exchanger function, the excess of ClC-3 was also found to increase the acidity of intracellular vesicles in NE tumor cell lines (BON, LCC-18 and QGP-1), thereby enhancing their resistance to the chemotherapeutic drug etoposide by almost twofold [97]. Because these cells seemed to be deficient in common multidrug resistance (MDR) transporters, the mechanism of enhanced drug resistance to etoposide was attributed to the ClC-3-mediated vesicular acidification, which represents a facilitating factor in vesicular drug sequestration.

Limitless replicative potential
Normal cells possess an intrinsic program that limits their proliferative growth to 60–70 divisions. The number of cell doublings is recorded within the terminal parts of chromosomes called telomeres. Telomeres consist of several thousand telomeric repeats of a short 6 bp sequence, which protect the ends of the chromosome [98]. During each cell division, the telomere is shortened by 50–100 bp, and the eventual loss of the telomere leaves chromosomal DNA unprotected, which causes chromosomal fusion, rearrangement and ultimately cell death. Thus, telomere shortening induces replicative senescence, which blocks cell division.
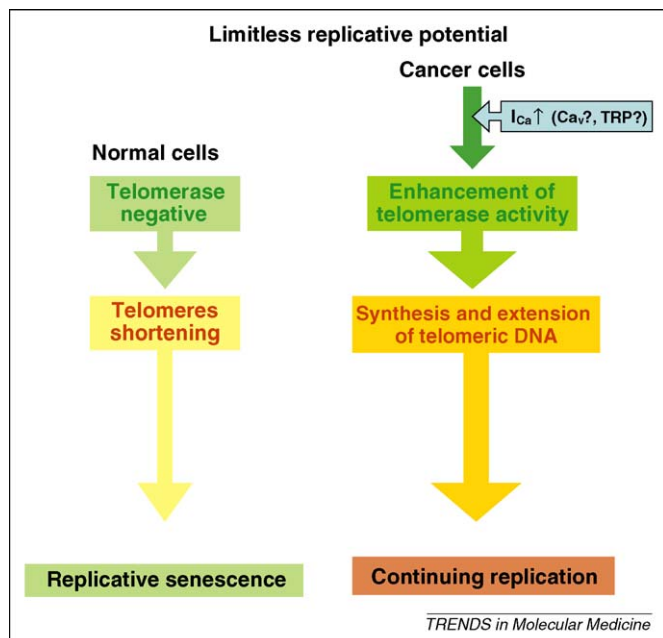
Figure 4. Limitless replicative potential. The prevention of chromosomal telomere shortening during the replication of cancer cells due to the enhanced activity of telomerase, which catalyzes the synthesis and extension of telomeric DNA and confers tumor cell immortality. Ca^{2+} influx ($I_{Ca}\uparrow$) via Ca_v channels or TRP members promotes the activation of telomerase.

By preventing telomere shortening, malignant cells can bypass cell division checkpoints and become immortalized. In 85–90% of cancer cells, telomere stabilization or extension is supported by telomerase, the multimeric ribonucleoprotein that catalyzes the synthesis and extension of telomeric DNA. An additional mechanism is based on the telomere–telomere recombination because of the activation of the alternative lengthening of telomeres (ALT) pathway [98]. Both mechanisms are strongly suppressed or nonfunctional in normal cells, which prevents limitless replication, whereas most tumors are characterized by the upregulation of telomerase activity (Figure 4).

Telomerase activity can be modulated by Ca^{2+} homeostasis. For instance, it has been shown that recombinant fungal immunomodulatory protein (reFIP-gts) exerts an anti-telomerase effect in A549 human lung adenocarcinoma cells that involves ER stress and intracellular Ca^{2+} release, which in turn prevents the nuclear translocation of the catalytic component of telomerase, human telomerase reverse transcriptase (hTERT) [99]. Moreover, in HaCaT human epidermal keratinocytes, the levels of telomerase activity were reduced in response to thapsigargin-induced ER Ca^{2+} release even without the downregulation of hTERT expression, suggesting that Ca^{2+} release can directly modulate the activity of the telomerase complex [100]. This modulation was postulated to occur via the S100A8 Ca^{2+}-binding protein, which inhibits the telomerase complex. An increase in Ca^{2+} levels, but not Mg^{2+} or Zn^{2+}, was also reported to reduce epidermal cell telomerase activity in a dose-dependent manner in a cell-free system [101].

Although released Ca^{2+} is likely to inhibit telomerase activity, Ca^{2+} influx might lead to the opposite effect. Indeed, the incubation of telomerase-positive SW626 ovar-ian carcinoma cells with elevated extracellular Ca^{2+} levels has been reported to enhance telomerase activity, and this effect was inhibited by verapamil, a blocking agent of L-type voltage-operated Ca^{2+} channels [102]. Thus, whereas the mechanism(s) of telomerase regulation is still poorly understood, the available data suggest that the spatial and temporal parameters of Ca^{2+} signaling might be important in determining the ultimate effect on telomerase activity and in turn on the replicative potential of the cancer cell (Figure 4). Further studies are needed to determine the potential importance of particular Ca^{2+} channels in these processes.

Sustained angiogenesis

The presence of oxygen and nutrients is crucial for sustaining cell function, growth and survival. Thus, to progress the neoplastic mass must develop an intrinsic capability to stimulate the growth of new blood vessels from the endothelium of the existing vasculature – a process known as angiogenesis. Although normal vascularization involves hierarchically assembled and efficient networks of blood vessels and capillaries, the vascularization of tumors can be largely chaotic, leaky and inefficient [103].

Activated vascular endothelial cells (ECs), whose proliferation and motility are controlled by different extracellular signals, are required for angiogenesis (Figure 5). Tumor cells secrete a number of growth factors that have mitogenic or pro-angiogenic effects on ECs *in vitro* and *in vivo*. These include vasoactive peptides, VIP, endothelin-1, angiotensin II, neuropeptide Y and calcitonin, as well as the vascular endothelial growth factor (VEGF) family of proteins, which are the most potent EC mitogens [104,105]. The VEGF proteins act on two specific receptor tyrosine kinases (RTKs) in the vascular system, namely VEGF-R1 and VEGF-R2, to stimulate new vessel growth as well as vascular permeability to water and large molecular weight proteins [104,105]. Other potent mitogens that bind to RTKs with vasospecific functions are fibroblast growth factors (FGFs) and platelet-derived growth factor (PDGF). VEGF, FGF and PDGF are released by several cell types, including ECs and tumor cells.

Ca^{2+} permeable channels

Ca^{2+} permeable channels and Ca^{2+}-dependent signaling are crucial for angiogenesis [106]. Carboxyamidotriazole (CAI), an orally active agent with antineoplastic activity, is an inhibitor of non-voltage-operated Ca^{2+} channels, acting via the disruption of Ca^{2+} channel-mediated signal transduction, and thereby causing the inhibition of VEGF signaling, endothelial proliferation and angiogenesis [107]. Using this compound it has been shown that Ca^{2+} entry is important for baseline and the FGF2-stimulated proliferation and invasion of human umbilical vein ECs (HUVECs) [108]. CAI treatment has also been reported to decrease the relative vascular volume in hepatic metastases of B16F1 murine melanoma cells by reducing the density and size of microvessels without affecting the vascular volume of normal liver tissue surrounding metastases [109]. Furthermore, CAI inhibits increases in $[Ca^{2+}]_i$ during VEGF-A-induced EC proliferation [107], and this is consistent with the key role of Ca^{2+} influx in angiogenesis.
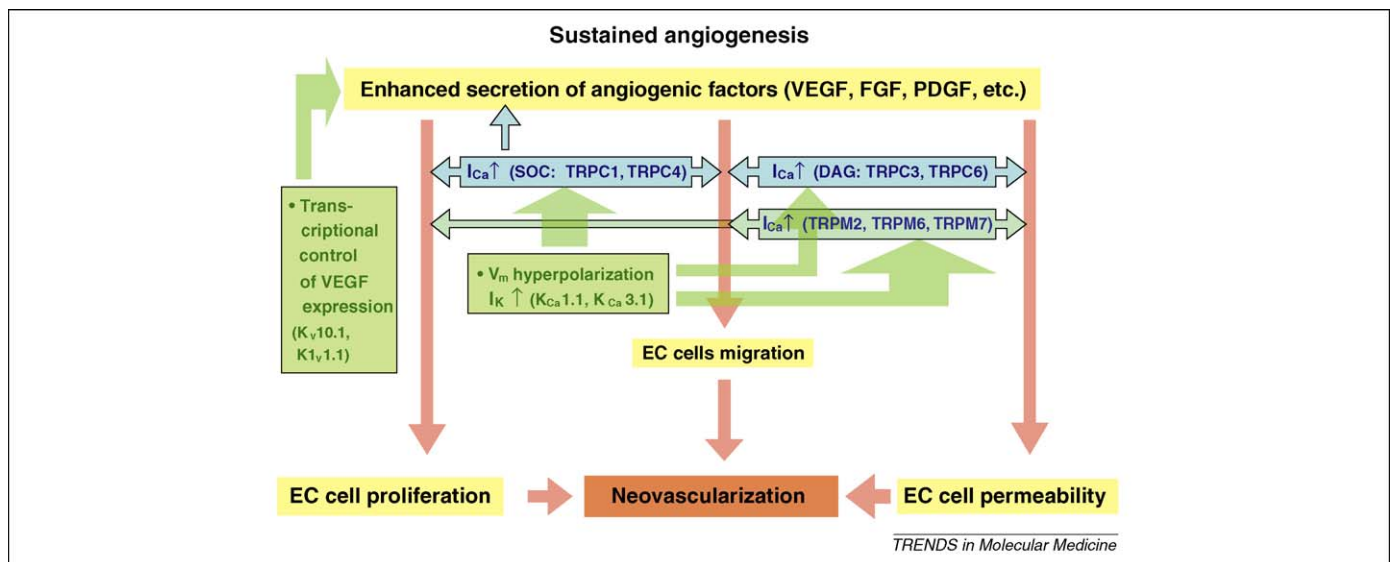
Figure 5. Sustained angiogenesis. The enhanced secretion of angiogenic factors by tumor cells promotes EC proliferation, migration and permeability change, which is crucial for the formation of blood capillary sprouts. Increased Ca^{2+} influx ($I_{Ca}\uparrow$) via store-dependent TRP members, TRPC1 and TRPC4, preferentially contributes to higher proliferation and migration rates of ECs, via DAG-gated TRP members, TRPC3 and TRPC6, to migration and permeability change and via redox state-sensitive, TRPM2, TRPM6 and TRPM7, most likely to all processes. Ca^{2+} influx via store-dependent TRPs also aids the enhanced secretion of angiogenic factors. The increased expression of Ca^{2+}-dependent K^+ channels ($K_{Ca}1.1$ and $K_{Ca}3.1$) facilitates membrane hyperpolarization, thereby promoting Ca^{2+} influx in a positive feedback manner. Oncogenic $K_v10.1$ and $K_v11.1$ are involved in the regulation of transcriptional VEGF production by tumor cells.

Because CAI seems to be specific for SOCs [110], abnormalities in the expression and/or function of molecular SOC constituents might be involved in enhancing the pro-angiogenic response of ECs in tumors. A number of store-dependent and -independent members of the TRP channel family are expressed in ECs [111], and these might contribute to the angiogenic process. In particular, TRPC1 and TRPC4 channels, which exhibit store-dependent gating, have been implicated in the Ca^{2+} influx required for the pro-angiogenic response of ECs. The involvement of DAG-gated TRPC3 or TRPC6 was implicated in the VEGF-induced permeability of microvascular ECs [112,113]. ECs also express redox state-sensitive members of the melastatin TRP subfamily (TRPM), TRPM2, TRPM6 and TRPM7 [111]; however, their involvement in neovascularization during cancer remains poorly defined. Thus, in addition to directly promoting EC proliferation, migration and permeability, Ca^{2+} influx through TRPs might stimulate ECs to produce and release the angiogenic growth factors VEGF, FGF and PDGF, which in turn might stimulate angiogenesis in an autocrine or paracrine manner.

K^+ channels

Ca^{2+}-activated K^+ (K_{Ca}) channels are thought to mediate endothelial hyperpolarization, which provides the electrochemical driving force for Ca^{2+} entry and the subsequent Ca^{2+}-dependent synthesis of vasodilating factors and gene expression (Figure 5). Studies of human mesenteric arteries from patients with colonic adenocarcinomas revealed an increased number of ECs expressing intermediate conductance (IK_{Ca} or $K_{Ca}3.1$) and large conductance (BK_{Ca}, Slo or $K_{Ca}1.1$) K_{Ca} channels without changing the average EC K_{Ca} membrane current density [114]. Augmented K_{Ca} channel expression in cancer patients resulted in a 2.7-fold enhancement of bradykinin-induced endothelial hyperpolarization compared with controls. It

was concluded that the enhanced expression and function of K_{Ca} channels might indicate an altered functional state of the endothelium in cancer patients and could play a role in tumor angiogenesis. Furthermore, the higher expression of K_{Ca} channels was detected in metastatic brain tumor tissue and tumor capillary endothelia compared with normal brain tissue [115]. K_{Ca} channel agonists and bradykinin selectively enhanced the permeability of the blood–tumor barrier (BTB) in brain tumors, but not in normal brains, thereby contributing to capillary endothelial leakage.

The pharmacological blockade of BK_{Ca} channels has also been shown to inhibit the FGF2-induced proliferation of HUVECs, whereas FGF2 was able to significantly potentiate BK_{Ca} channel activity [116], implicating the activation of BK_{Ca} channels in FGF-stimulated angiogenesis. However, in another study on the same type of ECs, although strongly augmented activity of BK_{Ca} channels was detected during VEGF-A- and FGF2-induced proliferation, the specific inhibition of these channels failed to impair proliferation [117], suggesting that the BK_{Ca} channel involvement in pro-angiogenic activities might be modulated by additional factors.

K^+ channels might be involved in angiogenesis not only via a feedback loop regulating V_m and $[Ca^{2+}]_i$ of ECs, but also by regulating VEGF production by tumor cells. Xenograft tumors induced in mice by the implantation of cells expressing the oncogenic Eag1 (Kv10.1, *KCNH1*) K^+ channel are characterized by excessive vascularization due to enhanced VEGF synthesis and secretion, which is associated with augmented transcriptional activity of hypoxia-inducible factor 1 (HIF-1) [118]. These effects, however, seemed independent of primary K^+-conducting Eag1 function, prompting the suggestion of "non-canonical" Eag1 interference with the HIF-1 control pathway under hypoxic intratumoral conditions. The specific pharmacological

blockade of human Erg1 (Kv11.1, HERG, *KCNH2*), which is overexpressed in high-grade gliomas, has been shown to decrease VEGF secretion by glioblastoma multiforme cells, most likely via the modulation of VEGF transcription levels [119]. It was concluded that the Erg1 channel might actively contribute to malignancy by stimulating neoangiogenesis typical of high-grade gliomas.

Tissue invasion and metastasis

Because of their high potential for migration, motility and invasion, tumor cells can penetrate blood or lymphatic vessels, circulate through the intravascular system and then proliferate at another site – the process termed metastasis. Cell migration, which underlies the metastatic phenotype, is a cyclical process involving the repetitive extension of invadopodia/lamellipodia at the leading edge of the cells, the formation of adhesion sites, the contraction of the cell body and the release of trailing adhesion sites. A number of PM ion channels, whose expression is altered in cancer, have been implicated in enhanced cell migration, motility and invasion, which are crucial for the formation of tumor metastases and progression of disease.

Na$^+$ channels

It has been shown that enhanced metastasis correlates with the appearance of membrane channels and currents that are characteristic of excitable membranes. This primarily involves voltage-gated sodium channels (VGSCs), whose enhanced expression has been detected in biopsies of metastatic breast, prostate and cervical carcinomas as well as in highly metastatic cancer-derived cell lines [120–122]. Interestingly, it seems that the presence of the Na$^+$ current rather than the excess of a specific VGSC protein is crucial for metastasis because in highly metastatic breast cancer cells the Na$_v$1.5 channel was found to be ∼1000-fold overexpressed [123], whereas in metastatic PCa tissues the Na$_v$1.7 channel is the most augmented (∼20-fold) [121]. Moreover, as shown in several non-small-cell lung cancer cell lines, the expression of multiple VGSC subunits *per se* is not sufficient for conferring an aggressive, invasive phenotype, and the presence of a Na$^+$ current is required for this phenotype to occur because it could be prevented by use of the specific VGSC antagonist tetrodotoxin [124]. The pharmacological blockade of VGSCs commonly results in the reduced migration of highly metastatic cell lines, whereas the facilitation of channel opening by agonists enhances migration without impairing cell proliferation or viability.

Structural and functional studies indicate that highly metastatic cancers mostly express embryonic isoforms of VGSCs [120,121,124], which supports the notion that human embryonic genes could be re-expressed in cancer cells [125]. The mechanisms responsible for VGSC upregulation and for their pro-invasive roles are still poorly understood. In hormone-responsive tissues such as uterus, ovaries, breast and prostate, VGSC expression might be under the control of steroid receptors. The additional control of the expression of the Na$^+$ channel might come from growth factors such as epidermal growth factor (EGF) and nerve growth factor (NGF), as shown for a number of highly malignant cancer cell types [126–128].

The importance of the Na$^+$ current rather than Na$^+$ channel type in determining the malignant potential of cancer cells is further highlighted by the expression in high-grade glioma cells of multiple members of non-voltage-gated Na$^+$ channels of the degenerin superfamily, ENaCs and ASICs. These channels are absent in normal astrocytes or low-grade tumor cells, and the inhibition of the Na$^+$ current that they carry decreases glioma growth and cell migration [129,130].

Hence, the overexpression of either voltage-gated (Na$_v$) or non-voltage-gated (ENaC, ASIC) Na$^+$ channels contributing to the altered electrophysiological properties of cancer cells or causing the perturbation of their intracellular ionic homeostasis represents an important factor in promoting cellular motility and metastasis (Figure 6).

K$^+$ and Cl$^-$ channels

Enhanced cell migration and metastasis also require the activity of K$^+$ channels (Figure 6), and in particular those that regulate cell V_m in response to G-protein-coupled receptor (GPCR) activation or changes in intracellular Ca^{2+}. Membrane potential in turn determines fluxes of key homeostatic ions Na$^+$, K$^+$, Cl$^-$ and Ca^{2+}, which control cell volume and intracellular signaling. In benign breast tissues, primary invasive breast carcinomas and metastatic breast carcinomas, a direct correlation was found between GIRK1 mRNA levels and metastatic behavior, and in particular with the number of lymph node metastases [51], whereas in the highly metastasizing MDA-MB-435 melanoma cell line (erroneously referred to as mammary cancer cells in the quoted study) the overexpression of small conductance Ca^{2+}-activated K$^+$ channels (SK3 or K$_{Ca}$2.3) was suggested to play a role in migration but not proliferation, potentially through involvement in the feedback loop regulating V_m and [Ca^{2+}]$_i$ [131].

The role of intermediate conductance Ca^{2+}-sensitive K$^+$ channels (IK$_{Ca}$ or K$_{Ca}$3.1) for sustaining cellular "migration machinery" was uncovered in Madin-Darby canine kidney (MDCK) cells, in which the pharmacological inhibition of these channels impairs cell migration [132]. It was found that the activity of IK$_{Ca}$ channels is primarily restricted to the rear part of migrating cells, which allows the localized volume loss that supports cellular retraction at the rear of the cell [132]. The cytokine FGF-2 has been shown to accelerate the migration of MDCK cells in an ERK1/2 phosphorylation- and IK$_{Ca}$ channel-dependent manner. It was suggested that FGF-2 exerts transcriptional control of IK$_{Ca}$ channels, which in turn participates in FGF-2-mediated signaling, leading to an acceleration of migration [133].

The HERG K$^+$ channel, whose enhanced expression is characteristic of a number tumor cell types, has been implicated in the invasive phenotype of colon cancer cells [134] because high levels of expression on both mRNA and protein were detected in metastatic human colorectal cancers, but not in normal colonic mucosa or adenomas.

The importance of K$^+$ and Cl$^-$ channels for supporting dynamic changes to cell shape and volume required for an increased capacity to move and invade through the narrow extracellular spaces is especially evident in glioma cells
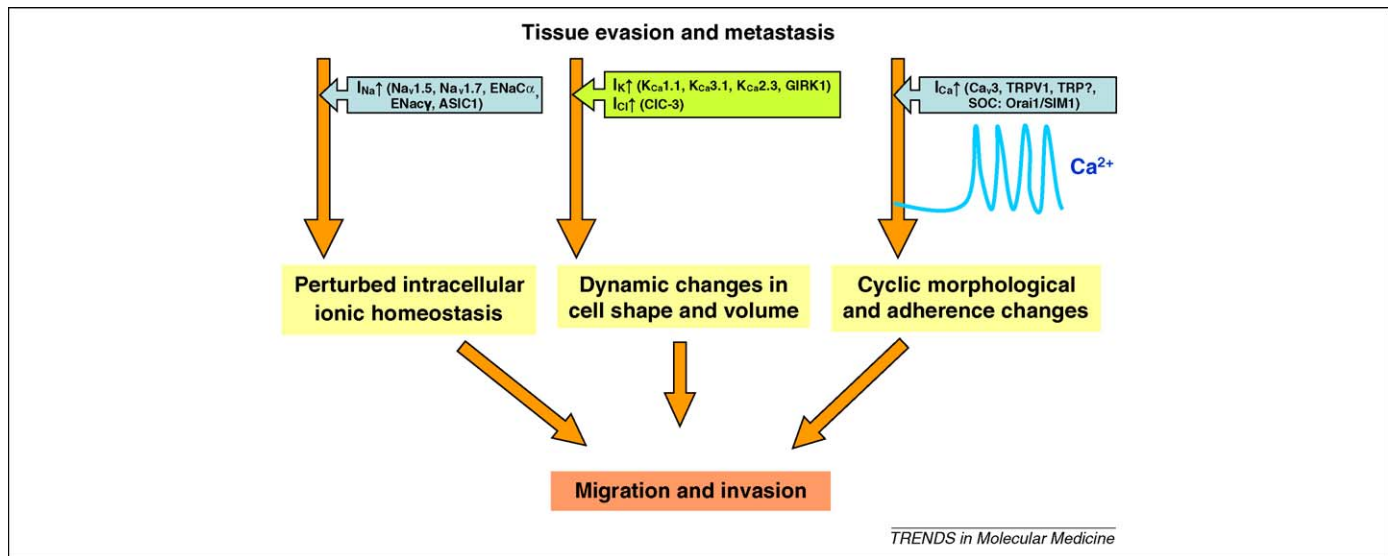
Figure 6. Tissue evasion and metastasis. The high propensity for the migration and invasion of tumor cells, which is crucial for metastatic behaviors, is determined by: 1) perturbed intracellular ionic homeostasis, 2) enhanced ability for dynamic changes of cell shape and volume and 3) ability for cyclic morphological and adherence changes. Augmented Na^+ influx ($I_{Na}\uparrow$) due to the overexpression of Na^+ permeable voltage-gated ($Na_v1.5$ and $Na_v1.7$) and non-voltage-gated (ENaC/ASIC) channels is the prime reason for perturbed intracellular ionic homeostasis. The overexpression of Ca^{2+}-dependent ($K_{Ca}1.1$, $K_{Ca}3.1$ and $K_{Ca}2.3$) and G-protein-regulated (GIRK1) K^+ channels together with the ClC-3 Cl^- channel enable augmented potassium ($I_K\uparrow$) and chloride ($I_{Cl}\uparrow$) fluxes, providing for more efficient dynamic control of cell shape and volume during migration and invasion. Cyclic morphological and adherence changes of metastatic cells involve periodic-type Ca^{2+} signaling supported by enhanced Ca^{2+} influx ($I_{Ca}\uparrow$) due to the overexpression of Ca_v3 and TRPV1, and might be some other TRP members as well as newly identified SOC constituents, Orai1/STIM1.

(Figure 6). In particular, the ClC-3 Cl^- channel and the BK_{Ca} ($K_{Ca}1.1$) K^+ channel have been implicated because these were shown to colocalize to lipid raft domains on invadopodia of glioma cells [13]. The inhibition of these channels impairs the cells' ability to migrate and limits tumor progression in experimental tumor models. More-over, the Cl^--channel inhibitor chlorotoxin [Cltx, a small, 36 amino acid neurotoxin isolated from the venom of the giant yellow Israeli scorpion (*Leiurus quinquestriatus*)] is currently in phase I/II clinical trials for the treatment of malignant gliomas [13]. Although the anti-invasive effects of Cltx on glioma cells are attributed to the toxin's inter-actions with surface matrix metalloproteinase-2 (MMP-2) [135], which are specifically upregulated in gliomas and related cancers but are not normally expressed in the brain, it is considered that such interactions might affect ClC-3 channels, indirectly inducing its internalization into caveolar rafts and eventually depleting it from the cell surface [13].

The involvement of Ca^{2+}-activated K^+ channels in pro-metastatic functions suggests a close association between Ca^{2+} signaling and volume regulation in conferring inva-sive cancer cell phenotypes. In PCa cells, Ca^{2+} entering the cell via SOCs has been shown to inhibit Cl^--permeable VRACs because of the colocalization of these channels in the confined caveola microdomains [136,137]. Such coupling is in part responsible for the enhancement of VRAC-mediated RVD capability in apoptosis-resistant PCa cells [94,95], which have reduced SOCE [83–85], and this might facilitate the dynamic changes in cell shape and volume required for migration.

Ca²⁺ permeable channels
The morphological and adherence changes observed during cell migration are accompanied by repetitive

changes in $[Ca^{2+}]_i$ in the form of Ca^{2+} spikes or oscillations [138]. However, the mechanisms by which Ca^{2+} permeable channels support such signaling are established only for limited cases of migrating cells and even less so for meta-static cancer cells (Figure 6). Based on the sensitivity of HT1080 fibrosarcoma cells' motility and invasion as well as the accompanying dynamic Ca^{2+}-signaling events to the T-type Ca^{2+} channel blocker mibefradil, and to the blockers of non-voltage-operated Ca^{2+} channels CAI and Gd^{3+}, it was concluded that the metastatic behaviors of these cells are dependent on certain types of Ca_v3 (most likely $Ca_v3.1$) and TRP (most likely TRPC1) channels [139]. The migration of human hepatoblastoma (HepG2) cells, which express two TRP channel family members, heat-sensitive TRPV1 and mechanosensitive TRPV4, could be essentially accelerated by the chemical agonist of the TRPV1 channel capsaicin, and inhibited by the TRPV1 antagonist capsazepine, but seem insensitive to TRPV4 impairment [140]. Just recently, newly identified molecular constituents for SOCs, STIM1 and Orai1 proteins (Box 1), have been implicated in the migration of vascular smooth muscle cells [141]. More-over, Orai1 and STIM1 seem essential for breast tumor cell migration *in vitro* and for tumor metastasis in mice; the siRNA-mediated reduction of Orai1 or STIM1 in highly metastatic human breast cancer cells or the treatment with a pharmacological inhibitor of store-operated calcium channels was shown to decrease tumor metastasis in animal models [142], highlighting the importance of SOCE and its new molecular components in normal and patho-logical cell migration.

Clinical implications and concluding remarks
Ion channels have emerged as important players in cancer-related processes. Although these studies are ongoing, there are signs of promising developments in

their clinical applications. CAI is a cytostatic inhibitor of non-voltage-operated calcium channels and calcium channel-mediated signaling pathways that suppress angiogenesis, tumor growth, invasion and metastasis, and is already in clinical trials as an orally active anti-neoplastic agent (http://clinicaltrials.gov/). In addition, a synthetically modified version of the Cl⁻-channel inhibitor chlorotoxin, iodine(^{131}I)-chlorotoxin or TM-601, is under investigation for the treatment of gliomas because of its specific effects on glial tumors but not normal cells (http://clinicaltrials.gov/).

However, to determine the importance of a specific type of ion channel as a potential therapeutic target for cancer treatment and diagnosis, several fundamental questions should first be resolved: 1) what is molecular nature of the channel; 2) in which specific type(s) of cancer is the channel over- or underexpressed; 3) in which cancer process(es) or hallmark(s) is the channel involved; 4) what are the endogenous signaling pathways that regulate the channel's activation; 5) which signaling pathways control channel expression; and 6) how specific is this channel to a particular type of neoplasm? Further progress in this field to answer such questions could facilitate the development of new anticancer therapies.

Because most channels are not cancer-specific, but rather are ubiquitously expressed in different tissues, their selective targeting in cancer cells remains the major challenge for therapeutic utilization because the pharmacological impairment of channel function is likely to produce significant toxicity to normal cells. A possible strategy for circumventing this problem might be the coupling of a drug to a targeting moiety to produce a drug derivative that can only be activated within the tumors. A good example of such a strategy is the so-called "smart bomb" for PCa that combines the sarco/endoplasmic reticulum Ca²⁺-ATPase (SERCA) inhibitor thapsigargin (which induces apoptosis via the activation of ER stress and Ca²⁺ entry pathways) with a targeting peptide representing the substrate for PCa-specific serine protease prostate-specific antigen (PSA) [143]. Such an inactive prodrug has been shown to demonstrate preferential toxicity only to PSA-producing PCa cells.

Additional complications in targeting channels for cancer therapies arise from the fact that selective pharmacological tools for many channels involved in cancer-related behaviors are simply unavailable. Therefore, new strategies targeting these channels based on siRNA and anti-sense technologies should be considered. Many ion channels are characterized by the presence of alternatively spliced variants, which might be differentially expressed in normal and cancer cells (such cases have already been described), providing the possibility of selectively influencing the channel with these technologies only in tumors while preserving its function in other tissues. Because ion channels are surface proteins, there might also be significant potential for antibody-based targeting, especially with respect to channels with a limited background expression but that are strongly overexpressed in tumors, such as Eag1. It has been shown that a specific monoclonal antibody that inhibits Eag1 function can effectively restrict cancer cell proliferation and reduce tumor growth in animal models with no significant side effects (i.e. neurotoxicity) [60]. In addition, even if anti-channel antibodies do not produce functional effects, they could be used as carriers for radionuclides, toxic molecules or nanoparticles.

The significance of ion channels is not limited to cancer therapies. As the expression pattern of the channels and the degree of their functionality change in cancer, they might be useful for diagnostic purposes. A good example of this is TRPV6, whose expression and function was shown to correlate with PCa grade [38,40]. The TRPV6 status of a tumor is a reliable predictor of clinical outcome: patients with TRPV6-positive PCa have a poor prognosis because of its higher potential for metastasis and tissue invasion beyond the prostate. An approach for PCa staging might also involve TRPM8 [26,27]. The decreased expression of the TRPM1 channel has been shown to correlate with melanoma cell transition from a low to high metastatic phenotype [44], whereas enhancement in tissue levels of oncogenic K⁺ channels (Eag1, Erg1 and TASK-3) might serve as an indication of malignant transformation [55,57–60].

The disadvantage of using channels as diagnostic tools is that they are membrane proteins that can only be detected in tissue biopsies and not in blood samples. However, this disadvantage could be an advantage for other detection methods because the use of fluorescently-labeled antibodies against oncogenic channels could allow tumor and metastasis sites to be visualized. This has been demonstrated for anti-Eag1 and xenografted tumors in live animals [60].

Ion channels still constitute a novel area of research in oncology. As this field is still rather young not all channel types have been investigated and for most of those studied the specific roles in different types of cancer are only just beginning to be understood. Moreover, the list of cancer hallmarks defined by Hanahan and Weinberg [1] has recently been extended by including new hallmarks such as "evasion of immune surveillance" [144] and stress-related phenotypes such as "metabolic stress", "proteotoxic stress", "mitotic stress", "oxidative stress" and "DNA damage-related stress" [145]. These additions have remained out of the scope of the present review, but with these and other developments in cancer biology, it is expected that a more detailed understanding of the roles of ion channels in the key processes involved in cancer will facilitate the development of improved molecular-targeted tools for diagnosis and treatment.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molmed.2010.01.005.

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