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Ion Channels and Cancer

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Abstract. Membrane ion channels are essential for cell proliferation and appear to have a role in the development of cancer. This has initially been demonstrated for potassium channels and is meanwhile also suggested for other cation channels and Cl^- channels. For some of these channels, like voltage-gated ether à go-go and Ca^{2+}-dependent potassium channels as well as calcium and chloride channels, a cell cycle-dependent function has been demonstrated. Along with other membrane conductances, these channels control the membrane voltage and Ca^{2+} signaling in proliferating cells. Homeostatic parameters, such as the intracellular ion concentration, cytosolic pH and cell volume, are also governed by the activity of ion channels. Thus it will be an essential task for future studies to unravel cell cycle-specific effects of ion channels and non-specific homeostatic functions. When studying the role of ion channels in cancer cells, it is indispensable to choose experimental conditions that come close to the in vivo situation. Thus, environmental parameters, such as low oxygen pressure, acidosis and exposure to serum proteins, have to be taken into account. In order to achieve clinical application, more studies on the original cancer tissue are required, and improved animal models. Finally, it will be essential to generate more potent and specific inhibitors of ion channels to overcome the shortcomings of some of the current approaches.

Key words: Cancer — Ion channels — K^+ channels — Cl^- channels — Proliferation — Cell cycle — Mitogens — Oncogenic — Apoptosis — Growth

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Content

Introduction

About 10 years ago, Wonderlin and Strobel summarized in their excellent topical review the role of potassium channels for proliferation and G1 progression [143]. Plasma membrane potassium (K^+) channels along with other ion channels belong to the fundamental equipment of any living cell and are required for cell proliferation. Over the past 10 years or so it became obvious that ion channels are crucial for tumor development and growth of cancer. During the change from a normal epithelial cell towards cancer, a series of genetic alterations occur, which may also affect the expression of ion channels or may cause a change in ion channel activity. This abnormal

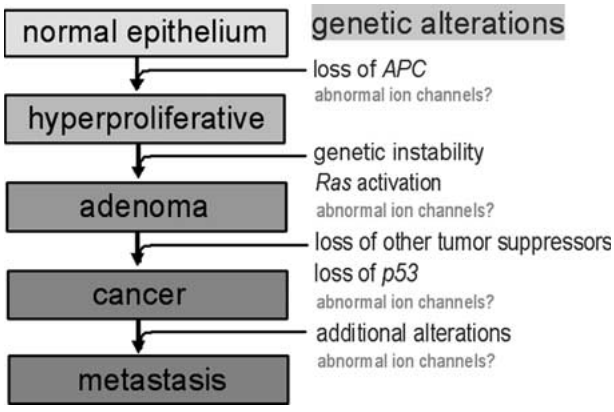

Fig. 1. Genetic alterations during development of colonic cancer.

ion channel activity is then able to support proliferation of the tumor (Fig. 1). It is neither clear at what stage of cancer development these channels appear, nor if these ion channels are even expressed differentially in the native cell and in the cancer. So far, most studies correlate the appearance of ion channels with proliferation of a cell line, but don't compare the properties of native epithelial tissues with that of cancer specimens. Conclusions are derived from proliferation studies, showing that inhibition of K^+ channel expression or channel blockade by more or less specific inhibitors reduces cell proliferation [137]. With an increasing number of studies it turned out that instead of a single type of K^+ channel being present in cancer cells from prostate, colon, lung, breast and other tissues, a variety of K^+ channels is found in these tissues, basically members of all known subfamilies of K^+-selective ion channels. The different subfamilies consist of Ca^{2+} -activated K^+ channels, Shaker-type voltage-gated K^+ channels, the ether à go-go (EAG) family of voltage-gated K^+ channels, as well as 2P-domain K^+ channels [87, 94, 137, 143]. These channels and their expression in cancer cells have been exhaustively reviewed by Wang [137]. Because different K^+ channels require very different cellular conditions to be functional, there is currently no clear understanding how these K^+ channels actually promote proliferation. In addition, not only K^+ channels have a role in proliferation but also other ion channels selective for Cl^-, Ca^{2+}, and nonselective cation channels. This short review summarizes the current knowledge of the role of these ion channels in proliferation and cancer development. Possible mechanisms for their proliferative effects are discussed along with their potential clinical use.

The Role of Ion Channels in Proliferation

Voltage-gated K^+ Channels

Studies on the role of voltage-gated K^+ channels (K^+ channels) for cell proliferation have been discussed in previous reviews [87, 94]. The well-examined voltage-gated ether à go-go (EAG) channel will be subject of a review by Pardo et al. in this special issue. Thus I will only concentrate on a few essential points, which may help to understand the concept of 'ion channels and proliferation'. Of all the different potassium channels found to have a role in proliferation, most studies are devoted to the impact of voltage-gated potassium channels on proliferation of tumor cells, particularly those of epithelial origin [3, 4, 10, 22, 35, 87, 94, 95, 98]. As far as the mechanism of tumor promotion is concerned, detailed work has been done on ether à go-go (EAG) K^+ channels, which turned out to be cell cycle-regulated K^+ channels [35, 94, 95]. The channel is inhibited upon onset of maturation, which is triggered by the mitosis-promoting factor (MPF). Moreover, intracellular Na^+ concentration and cytoskeletal interactions are essential for the inhibition [17, 21]. The majority of studies have been performed on cultured cell lines and only few studies compare the properties of ion channels in normal native epithelial tissue with those in carcinoma. Thus it is not really clear whether Kv channels are only expressed at significant levels in cancer cells, or whether they also have a role in the normal epithelial tissue [87]. Moreover, for most channels related to carcinogenesis it is not known at what stage of cancer development these channels actually appear. In non-excitable tissues significant expression of EAG channels was found only in cancer cells but not in normal control tissue [35]. Interestingly, other voltage-gated K^+ currents, which are present in myeloblastic leukemia cells, seem to vanish during differentiation and reduce proliferation [76].

Kv channels have a clear role in excitable tissues such as nerve and muscle cells of heart and other organs. Probably only in those cells, the membrane voltage will depolarize to a level that allows activation of Kv channels [87, 94]. This challenges the question, how these channels actually promote proliferation of epithelial cells. One important aspect is that the membrane voltage of cancer cells is typically more depolarized when compared to that of terminally differentiated epithelial cells [87, 94]. A depolarized membrane voltage (V_m) is detected when the cells are bathed in serum-containing media, but not in Ringer solution, i.e., when the cells are examined under proliferative conditions [12]. Thus, the depolarized V_m of carcinoma cells may provide the proper environment for activation of Kv channels [6]. Does this mean that Kv channels operate only in cancer cells but not in terminally differentiated cells, which are in the G_0 phase, or in non-carcinoma cells? Since almost all electrophysiological studies are undertaken on cells that have been removed from the affected organ and bathed in a Ringer-type solution, little is known about the membrane voltage of normal epithelial cells and

carcinoma cells in situ. The presence of serum proteins has pronounced effects on conductance properties and the membrane voltage. For example, T_{84} cells when studied under proliferative conditions, i.e., in the presence of culture medium, had a much more depolarized V_m than in Ringer solution [100] (Fig. 1*B*). In situ the basolateral membrane of epithelial cells is exposed to an interstitial fluid containing roughly 20–30 g/l protein and thus the membrane voltage may be more depolarized than in vitro [111]. Exposure to extracellular proteins and mitogens in the serum is probably even more important in the advanced stage of cancer, when the integrity of the basement membrane is disturbed and submucosal tissue and blood vessels are invaded by the cancer (Fig. 2*A*). Moreover, the particular composition of phospholipid metabolites in cancer cells and other intracellular factors may shift the activation window for Kv channels [87, 94, 134]. Finally, numerous other factors have to be taken into account, such as the completely different metabolic situation in the tumor tissue, the oxygen deprivation and the local acidosis. Taken together, we know little about the environmental impact on the activation of Kv channels and how these channels contribute to proliferation of cancer in situ.

Ca^{2+}-Activated K^+ Channels

Many types of tumors express Ca^{2+} -activated K^+ channels, such as those of the prostate [2, 12, 96], uterus [121], glial cells [7], stomach [34], pancreas [53], pituitary gland [26], breast [91] and colorectum [2, 71, 145]. Many reports are on cell lines rather than on the original tissue. Similar to the EAG channel, also for Ca^{2+}-activated K^+ channels, a cell cycle-dependent expression has been demonstrated. The density of the intermediate-conductance Ca^{2+}-activated K^+ channel hIK1 is enhanced in cells synchronized at the end of the G_1 and S phase and when compared with early G_1. This may in part be due to enhanced mRNA levels for hIK1. High hIK1 activity causes a highly negative membrane potential at the end of G_1 [91]. This negative membrane potential may support influx of Ca^{2+} and thus support the high basal cytosolic Ca^{2+} concentration in late G1. In contrast, expression of large-conductance (BK) K^+ channels was detected predominantly in the S phase of breast cancer cells. The impact of BK channel activation on proliferation is currently being evaluated [12, 90, 109]. In a recent study on prostate cancer cells we found enhanced expression of BK channels due to genomic amplification of the KCNMA1 locus encoding the α-subunit of the large-conductance Ca^{2+}-activated K^+ channel. Experiments show that this channel has a high activity in fast growing malignant prostate cancer cells, but shows only little contribution to the conductance in cultured epithelial cells from benign prostate hyperplasia [12] (Fig. 1*B*). Thus, convincing evidence exists that Ca^{2+}-activated K^+ channels control proliferation of cancer cells, probably during late G1 and S phase. It is unclear whether this finding applies only to cancer cells or whether the cell cycle of slowly proliferating cells is also determined by Ca^{2+}-activated K^+ channels. As outlined for voltage-activated K^+ channels, it is essential to prove that Ca^{2+}-activated K^+ channels are functionally relevant in cancer cells in situ. Thus, studies on tissues from healthy donors and patients with either benign hyperplasia or cancer are urgently needed.

Further evidence for the role of Ca^{2+}-activated K^+ channels in cell proliferation comes from studies on blood vessels. Large-conductance Ca^2-activated K^+ channels are important for endothelial cell proliferation and formation of atherosclerotic plaques [66, 141]. The proliferative properties of Ca^{2+}-activated K^+ channels have been closely linked to their influence on spatial and temporal organization of Ca^{2+} signaling in vascular smooth muscle and endothelial cells [84].

How much do large-conductance Ca^{2+}-activated K^+ channels contribute to the membrane voltage and cell proliferation? A study on different mammary epithelial cells only found a contribution of BK channels to cell proliferation when intracellular Ca^{2+} ($[Ca^{2+}]_i$) was elevated by intermittent stimulation with the agonist ATP [108]. Using the specific inhibitor iberiotoxin, we examined the role of BK channels in prostate cancer cells and only found a clear contribution of BK channels when the cells were examined in the presence of culture medium [12]. Culture medium is well known to increase $[Ca^{2+}]_i$. It may therefore be more appropriate to perform electrophysiological analysis in the presence of growth media. Along this line, another paper described activation of BK channels by the basic fibroblast growth factor [67]. Apart from BK channels, also intermediate-conductance Ca^{2+}-activated K^+ channels (IK1) were found to be essential for endothelial cell proliferation [44]. In contrast to BK, these channels are not voltage-dependent and thus Ca^{2+}-dependent activation may occur even at hyperpolarized membrane voltages.

Other K^+ Channels

One class of K^+ channels is characterized by the presence of two pore domains in tandem [97]. These so-called 2P-domain K^+ channels are open at resting membrane potential and are therefore regarded as leak or background K^+ channels. A member of this family of channels, the TWIK-related acid-sensitive K^+ channel TASK3 (KCNK9) is overexpressed in cancers from breast, lung and prostate due to genomic amplification [81]. KCNKL9 is genomically

amplified in biopsies of breast cancer and confers oncogenic properties, such as tumor formation, resistance to serum and oxygen deprivation [81]. Enhanced expression of this channel has also been detected in a large number of colorectal cancers, confirming the role of this channel in proliferation [60]. Although no specific inhibitors exist for this channel, it has been convincingly demonstrated that the proliferation-promoting effect and oncogenic potential of TASK3 depend on its K^+ channel function [99]. Moreover, the channel contains PKC- and PKA-phosphorylation sites, which provide regulatory opportunities beyond its background K^+-channel activity. Surprisingly, the same channel plays a central role during apoptosis of neuronal cells. As outlined by Patel and Lazdunski, the cellular context in which TASK3 (and probably other K^+ channels) operate, determines whether the channel is pro-apoptotic or oncogenic [97]. Another essential aspect concerns the amplitude of the K^+ current, which must be large to allow significant loss of cytosolic K^+ and cell shrinkage, both hallmarks of apoptosis. This point will be outlined below.

ATP-sensitive K_{ATP} channels are present in primary rat hepatocytes and human liver cell lines and were shown to promote proliferation of these cells [77]. Interestingly, Ca^{2+} fluxes were not affected by these channels and thus it was concluded that the pro-proliferative effects are not simply due to modulation of intracellular Ca^{2+}. Moreover, evidence exists for a role of K_{ATP} for G1 progression in human breast cancer cells [63]. Thus, various potassium channels are implicated in cell proliferation and cancer growth. The channels are very different regarding their activation, voltage dependence and single-channel properties. In many cancer tissues, such as human prostate and breast cancer cells, different K^+ channels such as Kv, BK, IK1 and K_{ATP} channels are expressed and all of them may have an effect on cell proliferation [2]. It is hypothesized that probably any type of K^+ channel has the potential to support cell growth, however, their contribution depends on additional cell-specific properties and environmental factors.

Lymphocytes, K^+ Channels and Immunosuppression

The role of K^+ channels for cell cycling and in particular for progression through G1 has been studied intensively for mitogenic activation of quiescent T lymphocytes. T cells are activated by phytohemaglutinin due to Ca^{2+} influx and transition from G0 to G1, expression of cell surface receptors for the cytokine IL-2, along with release of IL-2. Binding of IL-2 initiates a second Ca^{2+}-independent signal cascade that drives the cell from Gl to S phase. Margatoxin-sensitive Kv1.3 channels are most abundant in these cells, along with Ca^{2+}-activated K^+ channels [143]. Kv1.3 is crucial for production of IL-2 in early G1 and progression through the cell cycle. However, a rise in intracellular Ca^{2+} is essential for activation of T lymphocytes. The negative membrane voltage providing the necessary driving force for Ca^{2+} influx is provided by Ca^{2+}-activated IK1 (SK4) and voltage-gated Kv1.3 channels. After the initial activation of T cells, the message for the Ca^{2+}-activated K^+ channel hSK4 was much increased and the channel was shown to have a volume-regulatory function in those activated T lymphocytes [59]. Since blockade of these channels inhibits T cell proliferation and weakens the immune system, IK1 and KV1.3 have been proposed as novel therapeutic targets for autoimmune disorders [8]. Similar mechanisms have been described for maturation and proliferation of B cells. Mitogenic stimulation of these cells activates voltage-gated K^+ channels during G1 phase [5].

Cl^- Channels

Cl^- channels are expressed ubiquitously and a role of these channels in proliferation and cell cycling has been suggested in several reports. Unfortunately, many of the relevant Cl^- channels, like those activated by cell swelling and rise in intracellular Ca^{2+}, and the so-called outwardly rectifying Cl^- channel (ORCC) have not yet been cloned. A major hallmark of proliferating cells are Ca^{2+} fluctuations, which suggests that the activity of ubiquitously expressed Ca^{2+}-activated Cl^- channels changes during cell cycling. The family of CLCA proteins, originally discovered in bovine trachea, was claimed to form Ca^{2+}- activated Cl^- channels [25]. However, evidence is accumulating that these proteins are not Ca^{2+}-dependent Cl^- channels, but rather accessory proteins of Cl^- channels, formed by yet unidentified endogenous proteins [74]. Irrespective of these uncertainties, one member of the CLCA family, CLCA2, was suggested to mediate lung metastasis in conjunction with β_4 integrin [1]. In contrast, another paper reported pro-apoptotic and anti-neoplastic properties of CLCA1 and CLCA2. The message for these proteins was reduced in various tumor cell lines and a deleterious effect of CLCA on tumor cell survival has been demonstrated [32]. Since expression of both CLCA1 and CLCA2 was also downregulated in human colorectal cancer, these proteins were suggested to act as novel tumor suppressors [20].

Volume-regulated Cl^- channels were found in a human prostate cancer cell line and in lung cancer cells [56, 117]. Swelling-activated Cl^- currents have been associated with proliferation of mouse liver cells and angiogenesis [78, 142]. Inversely, downregulation of a volume-regulated anion conductance has been observed upon differentiation of muscle cells [130]. Outwardly rectifying Cl^- channels are activated through mitogen-activated tyrosine kinases in lymphocytes

[73]. Cl^- currents have a role in the proliferation of many cell types, such as microglia, glioma cells, cells from neuroblastoma and endothelial cells [40, 112, 119, 129]. Some members of the well described family of CLC Cl^- channels have also been associated with proliferation [55]. One study shows that the absence of CLC-3 Cl^- channel expression inhibits proliferation of vascular smooth muscle cells [132]. Cell cycle-dependent expression of Cl^- channels was observed, similar to that of K^+ channels. Thus ClC-5 expression in myeloid cells was high during S and G2/M but was low in G0/G1 [55]. In lymphocytes, Cl^- permeability also varied with the cell cycle, being low in G0 and S phase and high in G1/S [19]. The cell cycle-dependent expression of a glioma-specific Cl^- channel was linked to cytoskeletal rearrangements associated with cell division and cell swelling [126]. It is therefore likely that Cl^- channels balance transmembrane movement of ions and substrates during proliferation and provide a mechanism for regulatory cell volume decrease during cell cycling.

Ca^{2+} Channels and Other Cation Channels

Ca^{2+} is an essential regulator of the cell cycle and is indispensable for cell proliferation. Expression of voltage-gated L-type Ca^{2+} channels is enhanced in colonic cancer [136]. These changes are paralleled by enhanced epidermal growth factor signaling and Ca^{2+} increase in colonic cancer cells. T-type Ca^{2+} channels have been demonstrated as the capacitative Ca^{2+} entry pathway in cancer cells [43]. Voltage-gated Na^+ currents in rat prostate cancer cells determine lateral motility and invasive capacity in vitro and metastasis in vivo [36, 38, 45]. This is due to changes of the cytoskeleton, modulation of ion fluxes and regulation of gene transcription and enzyme activity. Similar effects were reported for T cells and it was shown that blockage of these channels by tetrodotoxin reduces invasiveness [37]. Apart from these voltage-gated channels, expression of amiloride-sensitive Na^+ channels was detected in malignant glioma cells [18]. Nonselective cation channels have also been associated with proliferation and cancer. Most important are ion channels of the transient receptor potential (TRP) family. They have been associated particularly with prostate cancer [140]. Their obvious role is to provide a Ca^{2+} influx pathway and probably depolarization of the membrane voltage, so that Ca^{2+} influx may occur through voltage-gated Ca^{2+} channels.

How Do Ion Channels Control Cell Proliferation?

Identification of Relevant Channels

Ion channel blockers are an important tool to determine the impact of ion channels on cell proliferation and cancer growth. However, pharmacologic approaches to identify crucial ion channels often face the problem of lacking specificity. Many of the ion channel blockers used, such as 4-aminopyridine (4-AP) and TEA^+, can enter the cell and cause additional uncontrolled effects, particularly when used at higher concentrations often necessary to inhibit the channel. Unfortunately, only a limited number of highly specific peptide-toxin inhibitors are available for a limited number of channels. A way around this problem is the use of small-interference RNA to reduce expression of respective channels. However, substantial suppression by siRNA is necessary to detect a contribution of the channel to proliferation. Moreover, the suppressed channel may be replaced by another conductance and thus the impact of channel protein may not be detectable.

As mentioned above, proliferation assays are done in the presence of growing medium, while functional measurements of ion currents, intracellular Ca^{2+} and pH are usually performed under serum-free conditions (Fig. 2). Culture media, however, depolarize the membrane voltage, induce uptake of substrates and trigger mitotic events, which may all affect ion channel activity. Thus, it is difficult to correlate proliferation with the activity of a particular ion channel. Moreover, since some ion channels show cell cycle-dependent expression, electrophysiological analysis of non-synchronized cells may deliver highly variable data. Another major concern is due to the fact that some potassium channel inhibitors affect the uptake of [$3H^+$]-thymidine, which is often used as a marker for proliferation [143]. In principle, ion channels may affect proliferation in two different ways: Any cell requires ion channel function to maintain basic homeostatic parameters, such as intracellular Ca^{2+}, pH and cell volume, and to allow uptake of substrates and release of metabolic products. Thus, inhibition of those channels will lower cell proliferation without interfering at a particular step during the cell cycle. On the other hand, ion channel activity, such as the hyperpolarizing activity of K^+ channels, is required at special check points during the cell cycle and therefore will have a precise role in proliferation.

Cell Cycle

Developing embryos and dividing cells have internal clocks or timers, which coordinate development and cell division. "Hourglass" timers are based on the decay or accumulation of cellular products up to a critical concentration, while "clock-like" timers oscillate and show a feedback control by environmental factors [57]. These timers are the basis for the cell cycle that is controlled by cyclin-dependent kinases and oscillating cyclins (Fig. 3). The activity of K^+, Ca^{2+} and Cl^- channels changes during cell

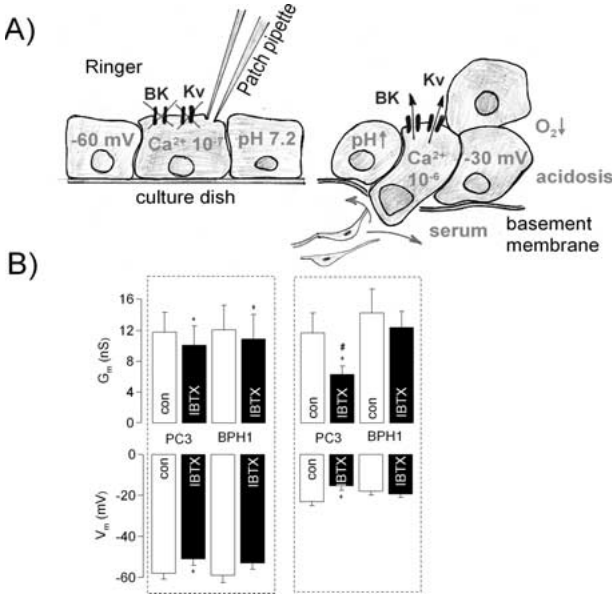

Fig. 2. Impact of environmental conditions during patch-clamp experiments and in vivo on ion channel activity. (*A*) Large-conductance BK and voltage-gated Kv channels may not be active in cultured epithelial cells in the presence of a Ringer-type bath solution. The same channels are active in the intact tissue due to cytosolic alkaline pH, increase in intracellular Ca^{2+} and depolarized membrane voltage. (*B*) Whole-cell membrane conductance (*G*m) and membrane voltage (V_m) obtained from benign prostate epithelial cells (BPH1) and malignant prostate cancer cells (PC3) in patch-clamp experiments. The left group of bars shows results obtained in the presence of Ringer bath solution, the right group of bars shows results obtained in the presence of culture medium as the extracellular bath solution. It is clearly shown that both types of cells are hyperpolarized in the presence of Ringer solution, but are strongly depolarized in the presence of medium. Inhibition of BK channels in the cancer cells by iberiotoxin (IBTX, 30 nM) was much more pronounced in the presence of medium. *Indicates significant ($p < 0.05$) differences when compared to control (paired *t*-test). #Indicates significant ($p < 0.05$) differences when compared to Ringer bath solution (unpaired *t*-test); $n \geq 10$ for each series of experiments.

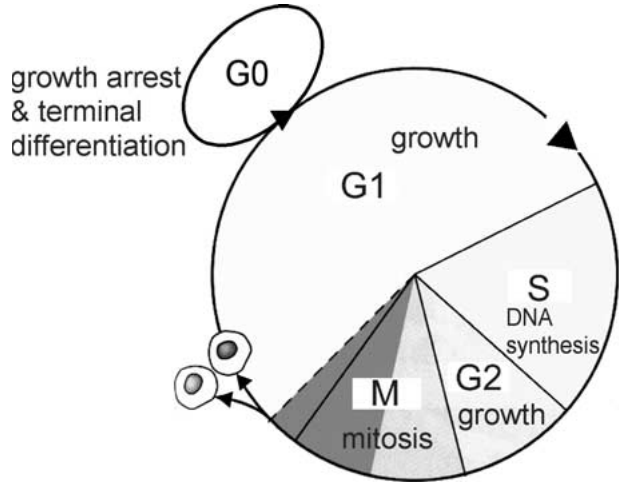

Fig. 3. Schematic drawing of the cell cycle.

cycling and this channel oscillation itself may be a result of cell cycling or may be part of the clock mechanism, together with nucleus-independent cytoplasmic oscillators and the fluctuating activity of kinases [13, 28]. There is good reason to assume that the cycling of channel activity is not only an epiphenomenon of the cell cycle, but rather necessary to drive the oscillatory rhythm. Cell cycle-dependent oscillatory activity of EAG/ERG-like K^+ channels, which is high in M/G1 and low in S/G2, appears independent of the nuclear function and cyclin-dependent kinase [29]. In *Xenopus* oocytes the EAG channel is inhibited at the G2/M transition, thus inducing depolarization of the membrane [17]. Inhibition of the current is due to the mitosis-promoting factor, which is a complex of cyclin B and $p34^{cdc2}$. EAG contains consensus sites for mitogen-activated protein (MAP) kinases as well as mitosis-promoting factor (MPF), also known as cyclin-dependent kinase 1 [21]. K^+ channels of the EAG family also contain an EH-binding domain for the cell cycle-regulating protein epsin [101]. Moreover, the channel is inhibited by intracellular Na^+, which shows a continuous change in concentration during the cell cycle. Eventually, the dramatic changes in the microtubular cytoskeleton at the beginning of mitosis leads to rectification of the ion channel current [21]. On the other hand, inhibition of K^+ currents and membrane depolarization cause accumulation of the cyclin-dependent kinase inhibitors p27 and p21 [41]. Thus, cell cycle-relevant proteins may be directly regulated by membrane voltage. Apart from this cell-cycle dependence, EAG expression continuously increases during mouse embryonic development from the 8-cell stage towards development of the morula [139].

As outlined above, EAG, large (BK)- and intermediate (IK1)-conductance K^+channels and K_{ATP} channels drive progression through G1 and cause fluctuations in the membrane voltage [144]. According to presently available data, a model may be proposed in which EAG and BK dominate in early G1 when the membrane potential is fairly depolarized, allowing activation of both channels. Hyperpolarization towards G1/S increases $[Ca^{2+}]_i$, which gates IK1 channels. These changes may be paralleled by cell cycle-dependent expression of voltage-gated L- and T-type Ca^{2+} channels and voltage independent Ca^{2+} influx pathways in excitable and non-excitable tissues, respectively [65]. T-type Ca^{2+} channels are large in unfertilized oocytes and drop deeply towards the M phase. These changes have been found to be independent of cdkl/cyclinB [27]. Finally, even Cl^- channels, such as the voltage-activated Cl^- channel, are regulated by the cell cycle clock [13, 128]. These channels showed highest

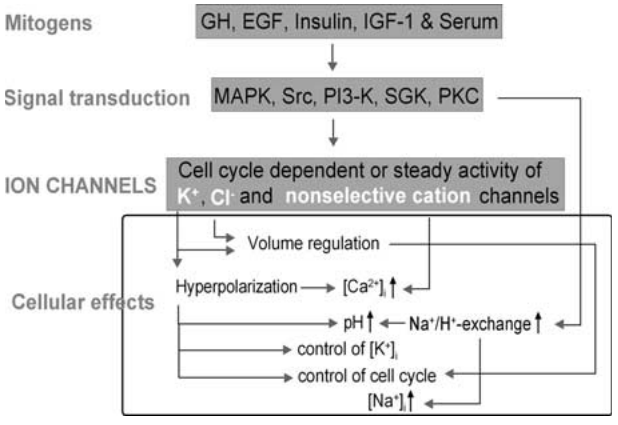

Fig. 4. Schematic drawing of the cellular effects of ion channels in a proliferating cell. Mitogens like growth hormone (GH), epithelial growth factors (EGF), insulin, insulin-like growth factor 1 (IGF-1), and exposure to serum trigger intracellular signal cascades. Signal transduction by mitogen-activated protein kinase (MAPK), tyrosine kinase (Src), phosphatidyl inositol kinase (PI3-K), serum and glucocorticoid-dependent kinase (SGK) and protein kinase C (PKC) activates ion channels and the Na^+/H^+ exchanger. Cell cycle-dependent or steady activation of ion channels activated by cellular signal transduction or due to de novo channel expression induces various cellular effects as shown.

activity after fertilization. Other examples comprise ClC-5 expression in myeloid cells (high in S and G2/M, low in G0/G1), the Cl^- permeability in lymphocytes (high in G1, low in G0 and S) and glioma cells (high in Gl) [19, 55, 126].

Growth Factors and K^+ Channel Activity

Growth factors such as insulin, IGF-1, and EGF stimulate cell proliferation. These growth factors transmit mitogenic signals through binding to and activation of their cognate receptors. Stimulation by growth hormones induces receptor dimerization and activation of the associated tyrosine kinase, Janus kinase Jak2. Transphosphorylation of Jak2 and activation of the transcription (Stat) proteins are paralleled by activation of the Ras/mitogen-activated protein (MAP) kinase and the phosphatidyl-inositol-3′-(PI3) kinase pathways [58]. The signals, in the form of reversible phosphorylation, are transduced and amplified through downstream kinase cascades, inducing cell survival, growth, differentiation, and metabolic changes. The Raf-MEK-ERK axis of proteins resembles a major growth factor-mediated signaling pathway. ERK1 and ERK2, the terminal kinases in this module, regulate a myriad of substrates important for growth and differentiation (Fig. 4).

K^+ channel modulation by mitogenic, i.e., growth factor-mediated signaling, seems to be a general phenomenon. Mitogenic stimulation of resting cells in the G0 phase activates K^+ channels and drives the cells into G1, thus initiating proliferation. In HEK293 cells, IGF-I upregulates expression of several Kv channels as part of the mitogenic response. The hormone prolactin stimulates cell proliferation through a tyrosine kinase-dependent increase in K^+ channel open probability [127]. In ras-transformed cells, linear hyperpolarizing K^+ currents generated by K_{ATP} channels were significantly larger than in untransformed cells [64]. Mitogenic stimulation activates Kv channels, which are required for progression through the cell cycle [47, 107]. Interestingly, K^+ channel activity appears to be an upstream modulator of growth factor-mediated ERK and Akt pathways. As demonstrated in some cell types, the enhanced K^+ conductance causes K^+ efflux and the resulting membrane hyperpolarization leads to transient Ca^{2+} influx and cell swelling. The currently available results suggest an early involvement of K^+ channels in the initial mutagenic signal events at the membrane level. Moreover, K^+ channel activation may lead to receptor clustering, thus facilitating transmembrane signaling.

Voltage-gated Kv1.3 channels are activated by IGF-1 through PI3 kinase (Fig. 4). Interestingly, like several other ion channels, KV1.3 is regulated by the serum- and glucocorticoid-inducible kinase SGK1 [39, 48]. SGK1 is activated through PI3 kinase and inhibits the ubiquitin ligase Nedd4-2, thereby inhibiting channel endocytosis. These recent findings could explain why so many different ion channels are activated through mitogenic stimulation, because SGK1 regulates a broad spectrum of channels [70]. There is good reason to postulate activation of several ion channels through this pathway in cultured cells, since culture medium typically contains 1–10% serum. Thus, cells exposed to serum respond with activation of potassium currents as well as of cation channels permeable to both Na^+ and Ca^{2+} [75].

The Importance of the Membrane Voltage

The importance of the K^+ channel-induced negative membrane voltage for cell proliferation has been shown in many studies. There is a clear correlation between membrane potential and mitotic activity. Thus, terminally differentiated cells in G0 phase are very hyperpolarized, rapidly cycling cells, such as tumor cells never entering G0, are very depolarized, and quiescent cells that need to be activated by mitogens to re-enter the cell cycle, are somewhere in between [11]. Mitogenic stimulation induces a short hyperpolarization peak at early G1, followed by depolarization. Although subsequent hyperpolarization during G1 has not been reported for all cell types, it is frequently observed and is believed to be essential for proliferation. Notably, the apoptosis-inhibiting protein Mcl-1 has been demonstrated to cause membrane hyperpolarization by activation of

K^+ channels [135]. Mcl-1 plays a critical role in controlling life and death decisions in response to rapidly changing environmental conditions. It is also required for embryonic development of the immune system. Taken together, maintaining the proper membrane voltage during cell cycling is of fundamental importance and is controlled by K^+ channels. As outlined below, both depolarized and hyperpolarized membrane voltages are crucial for proper Ca^{2+} signaling and appear to affect regulation of the cytosolic pH. Apart from these homeostatic tasks, the hyperpolarized membrane voltage drives the transport of substrates. Thus, Na^+-coupled uptake of nutrients is driven by the electrochemical gradient for Na^+, which is in part maintained by the negative membrane voltage [143]

Ca^{2+} Signaling

Ca^{2+} signaling is fundamental for cell cycling and proliferation. It requires Ca^{2+} release from intracellular stores and Ca^{2+} entry from the extracellular space, through different types of Ca^{2+} channels, depending on the cell type. In excitable tissues, Ca^{2+} influx occurs through voltage-gated Ca^{2+} channels. Ca^{2+} increase associated with proliferation in pulmonary artery smooth muscle cells is due to cell cycle-controlled inhibition of Kv currents, which leads to membrane depolarization and activation of voltage-gated Ca^{2+} channels [102]. In non-excitable tissues hyperpolarization of the membrane voltage is important for the increase of intracellular Ca^{2+}, providing the driving force for Ca^{2+} entry from the extracellular space. Moreover, Ca^{2+} is highly concentrated in intracellular stores and is released from the endoplasmic reticulum or mitochondria upon mitogenic stimulation [72, 85, 86]. The various influx and release pathways for Ca^{2+} are described in detail by Schreiber in this issue. Proliferative Ca^{2+} increase upon mitogenic stimulation or through oncogene action is probably more complex, i.e., the Ca^{2+} signal may oscillate and influx of Ca^{2+} changes continuously during progression through the cell cycle [68, 92].

Regulation of Cytosolic pH

Cytosolic pH fluctuates in a cell cycle-dependent manner in many cell types. An increase in cytosolic pH parallels the rapid and transient rises in $[Ca^{2+}]_i$ upon binding of growth factors and during G 1/S transition. pH rises over a range of 0.2 – 0.4 units during Gl and drops briefly before S. This is caused by an increase in the activity of the Na^+/H^+ exchanger NHE1, which is activated due to mitogenie stimulation, involving PKC activation and actin depolymerization. NHE1 exchanges intracellular H^+ for Na^+ from the extracellular space. Thus, alkalinization is driven by Na^+ uptake through the chemical gradient for Na^+. As a consequence of the enhanced Na^+/H^+ exchange, the Na^+ concentration increases, which turns on the Na^+/K^+ ATPase. The ATP-consuming Na^+/K^+ ATPase lowers $[Na^+]_i$ and accumulates intercellular K^+ [79]. Finally, K^+ ions need to recycle through membrane K^+ channels in order to keep the Na^+/K^+ ATPase going. As a result of this transport, $[Na^+]_i$ is low in early G1 and S phase and high during late G2/M and these changes are paralleled by similar fluctuations in $[K^+]_i$ [80]. Inhibition of K^+ channels may affect regulation of cytosolic pH and reduce proliferation by causing cellular acidification. In fact, we found a reduced realkalinization from H^+ load after blocking K^+ channels along with inhibition of proliferation (*unpublished data*).

The necessity for proper control of intracellular pH in proliferating cells has been known for a long time. A hypoxic and acidic microenvironment is formed by highly proliferative cancer cells, producing large amounts of metabolic acid due to glycolysis, glucose utilization and lactic acid production. However, the responsible enzymes operate at slightly alkaline pH and the cells cope with the acid load and maintain an alkaline intracellular pH by increasing proton efflux, which would otherwise lead to apoptosis. Regulators of cellular pH, such as the proton pump, the Na^+/H^+ exchanger HCO_3^- transporters and monocarboxylate transporters, show enhanced activity in cancer cells [52]. Stimulation by growth factors induces tyrosine phosphorylation and sustained increase in intracellular pH by activation of the Na^+/H^+ exchanger NHE1 [131]. The concept of intracellular alkalinization as an early event during malignant transformation has been demonstrated in many cell types [106]. However, intracellular alkalinization inhibited proliferation of astrocytes, which was surprisingly induced by K^+ channel blockers [93]. Moreover, cellular alkalinization was observed during inhibition of volume-regulated anion channels. These channels are activated during apoptotic cell shrinkage and have a role in apoptotic cell death. However, swelling-activated Cl^- channels also have proliferative function, depending on the overall cellular and metabolic situation.

Volume Regulation

During progression through the cell cycle, the cell volume continuously changes: The cytosol accumulates substrates, proteins are synthesized and DNA doubles. Cellular growth, mitosis and cellular migration necessarily perturb the cell volume and thus mechanisms need to be in place to compensate for these imbalances. Particularly during G1/S transition and around the M phase large volume changes occur. There is a clear correlation between cell

volume and rate of proliferation. On one hand, mitogens stimulate uptake of substrates and cell swelling; on the other hand, swelling activates regulators of the cell cycle such as ERK-1 and ERK-2 [30, 110]. The cytosol behaves more like a gel than a fluid compartment. It is rather crowded due to cytosolic structures and tightly packed proteins competing for hydration. The activity of macromolecules such as enzymes is largely dependent on their concentration [30, 33]. Thus, changes in cell volume affect the rate of enzymes controlling cell proliferation. In fact, there is a bell-shaped correlation between cell size (volume) and proliferation. Excessive activation of either K^+ or Cl^- channels inhibits proliferation and leads to apoptotic cell death.

Rapidly proliferating tumor cells migrate and have a high metabolism and enhanced mitotic rate in comparison with cells in growth arrest at G0. Thus, a regulatory volume decrease (RVD) is in place, due to parallel activation of K^+ and Cl^- channels. These additional channels improve RVD, which helps cells to survive the harsh changes occurring during rapid proliferation. Cl^- channels take also part in the regulatory volume decrease and are expressed in a cell cycle-dependent manner. This has been demonstrated for the volume-regulated anion channel (VRAC) in human cervical cancer cells [115]. Arresting the cells in the G0/G1 phase was accompanied by a significant loss of VRAC activity. These volume-regulated Cl^- channels of unknown molecular nature may not only control cell volume. Uptake of amino acids and other substrates for DNA and protein synthesis occur, at least in part, by these channels [23, 62, 143]. The idea of an enhanced volume regulatory capacity in tumor cells was put forward by the observation that the oncogene BCL-2 augments swelling-induced Ca^{2+} entry and activation of Cl^- channels [116]. However, as outlined below, activation of volume-regulating K^+ and Cl^- channels beyond a certain level will cause cell shrinkage and promote apoptosis.

Do Ion Channels Determine Malignancy and Metastasis?

After all, and on the basis of the available information, this question is difficult to answer. A considerable body of evidence exists that additional K^+, Cl^- and Ca^{2+} channels in tumor cells support their proliferation. It is currently not clear if any hyperpolarizing K^+ conductance is able to support progression through G1 or only selected ones. We may suggest that any small additional K^+ conductance supports proliferation, because i) the background K^+ conductance generated by TASK3, ii) a large variety of K_{ATP}, Kv and Ca^{2+}-activated K^+ channels, and iii) even low concentration of the K^+ ionophore valinomycin, which serves as an artificial K^+ channel, all enhance proliferation ([120] and our own unpublished data). On the other hand, constant hyperpolarization could be detrimental for proliferation, since Ca^{2+} signaling and other voltage-sensitive events only occur at particular steps during cell cycling.

The complex process of carcinogenesis is triggered by oncogenic pathways, several of which have been shown to include activation of K^+ channels. For example, p21ras and its immediate downstream target, the Raf kinase, are known to induce oncogenic transformation and have been shown to activate Ca^{2+}-dependent K^+ channels. Oncogenic transformation of cells by either *ras* [49], Rous sarcoma virus [105] or SV-40 virus [125] is paralleled by enhanced K^+ channel activity. The importance of the K^+ channel in non-transformed cells for mitogenic signaling was further demonstrated by stimulation through epidermal growth factor and platelet-derived growth factor [49]. For the Src tyrosine kinases a direct phosphorylation and activation of Kv channels was demonstrated [118]. Taken together, a role of K^+ channels during oncogenic transformation and malignancy can be suggested. This is clearly supported by the study of Pardo et al. [95] demonstrating the oncogenic function of the EAG channel under in vitro and in vivo conditions. Enhanced cell migration and metastasis indicate malignancy and also require K^+ channel activity. E.g., intracellular Ca^{2+} is increased during intestinal wound healing and Kv channels are activated [103, 114]. Cell migration and formation of tumor metastasis is due to fluctuations in the activity of membrane transporters and ion channels. They cause localized cell swelling and shrinkage, a process that has been well described by Schwab [113].

The Paradox of Apoptosis and Proliferation

Programmed cell death named apoptosis is initiated by death-promoting molecules such as TNF-α and CD95 Fas ligand, and was directly correlated to an early activation of K^+ currents and loss of intracellular K^+ [137, 147, 148]. As a consequence, apoptotic cells show apoptotic volume decrease and shrink. Subsequently, metabolic enzymes are activated, such as caspases and nucleases, which further propagate death signals. Remarkably, all these enzymes are controlled by the intracellular K^+ concentration and while the concentration of various ions may change during apoptotic cell shrinkage, that of K^+ plays a necessary and probably pivotal role in the cell death program [14, 15, 51]. Moreover, K^+ efflux and apoptotic cell shrinkage may be triggered by various types of K^+ channels; some of them are even located in the mitochondrial membrane [46, 104, 122, 137].

Paradoxically, the same type of Ca^{2+}-activated K^+ channel that promotes cell proliferation, is also activated during apoptotic cell shrinkage [88]. Activation of volume-regulatory K^+ and Cl^- ion

channels under normotonic conditions results in cell shrinkage below the normal cell volume and precedes cytochrome c release, activation of caspase 3 and DNA laddering. Along the same line, HERG channels promote H_2O_2-induced apoptosis of various tumor cells and also facilitate tumor cell proliferation by tumor necrosis factor α [133]. These and other reports ask for reconciliation of these apparent contradictions. Apoptosis is triggered by activation of death receptors by death-promoting molecules (exogenous trigger) or mitochondrial damage (endogenous trigger). These and numerous other events take place during initiation of apoptosis, which are not observed during proliferation. Although cell shrinkage belongs to the early events in apoptosis, volume decrease is not the very first event but is preceded by reduction in glucose uptake, protein synthesis and MAP kinase activity [146]. The opposite is observed during proliferation (Fig. 5).

Activation of K^+ channels during apoptosis is much more pronounced than during proliferation and leads to cell shrinkage [146]. Moreover, K^+ efflux during apoptosis largely exceeds that observed during K^+ channel activation in cancer cells. This leads to a drastic fall in the intracellular K^+ concentration to 50 mM and even below [50]. In contrast, during cell cycling, $[K^+]_i$ oscillates around higher concentrations [124]. In general, high intracellular K^+ concentrations are found during mitosis, while terminal differentiation (G0 phase), e.g., of myeloid leukemia cells, is characterized by reduced intracellular $[K^+]_i$ and increased $[Na^+]_i$. Since many of the growth-and mitosis-related enzymes require a minimal $[K^+]_i$, a loss of K^+ will reduce the proliferative activity. Moreover, lowering the intracellular Cl^- concentration by activation of apoptotic Cl^- channels will also trigger programmed cell death. Both Cl^- and K^+ efflux are tightly coupled and take place during AVD. Thus, K^+ loss, Cl^- loss and AVD are closely related and each is able to trigger apoptosis. Several types of Cl^- channels have been demonstrated to participate in cellular apoptosis. These channels comprise the cystic fibrosis transmembrane conductance regulator (CFTR) [42], the outwardly rectifying Cl^- channel (ORCC) of unknown molecular identity [123], Ca^{2+}-activated and volume-sensitive Cl^- channels, which have not yet been cloned [61, 89, 116]. Despite the parallel activation of Cl^- and K^+ channels, K^+ loss exceeds that of Cl^-, since K^+ movement can also be balanced by other intracellular anions. Thus, Cl^- movement is probably closely associated with cell volume regulation, while K^+ homeostasis is the more critical regulator of apoptosis [138]. From the present findings one may conclude that activation of both Cl^- and K^+ conductances must stay within a certain conductance range to support proliferation, otherwise programmed cell death is triggered [69].

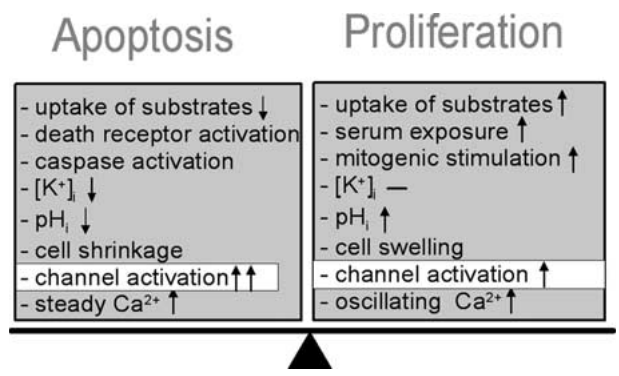

Fig. 5. Schematic drawing of various factors that differ between apoptosis and proliferation. Environmental factors along with differences in the ion current amplitude determine whether ion channels support apoptosis or proliferation.

Apart from these obvious differences between differentiation and apoptosis, other important factors could be of importance, such as the differences in Ca^{2+} signaling. Oscillatory Ca^{2+} rises were associated with proliferation and have not been observed during apoptosis [9]. A more steady Ca^{2+} increase, however, appears to be necessary for activation of apoptotic enzymes. Although we still have no complete and satisfying image of the dual effects of K^+ and Cl^- channels, it is probably fair to say that the environmental conditions in which channel activation takes place, along with the magnitude of the activated conductance, essentially determine whether the channel supports proliferation or apoptosis (Fig. 5).

Close to a Clinical Application?

Considerable evidence exists for the contribution of ion channels to development and growth of cancer and malignancy of the tumor, yet a clinical application of this knowledge appears to be far away. This may have several reasons. On one hand, our understanding of the role of K^+ channels and other ion channels for cell proliferation and apoptosis is incomplete. On the other hand and as outlined above, very little knowledge exists regarding the functional impact of the channel in the real tumor tissue and the role of these channels at different stages of tumor development. Finally, we still lack highly specific blockers for many of the ion channels related to cancer, like the BK K^+ channel or Kv channel.

Abnormal expression of ion channels could be used as marker proteins for malignant transformation, but only if the particular channel is expressed differentially in the normal tissue and the carcinoma. Thus, expression of the channel could serve as a prognostic parameter (*see* the review by Schönherr in this issue). Highly specific toxins for inhibition of ion channels are certainly the gold standard, but only available for some types of K^+ and Cl^- channels.

Specific blockers exist for large-conductance BK channels, such as charybdotoxin, iberiotoxin or paxillin, while intermediate-conductance channels are inhibited by charybdotoxin and Tram-34 [54]. Blockers of the Ca^{2+} entry pathway may also inhibit proliferation. They may act indirectly by inhibition of K^+ channels (reduced driving force), or by directly inhibiting cytosolic Ca^{2+} rises (Ca^{2+} channel blocker) essential for cell cycling and proliferation [83]. Except for very few compounds, most of the Kv channel blockers are fairly non-specific. Indirect inhibitors of Kv channels have been described, such as the PI3-kinase inhibitor LY294002, which, however, also block various types of Kv channels [31]. Potent and specific inhibitors of two-pore domain channels are currently not available. E.g., the antidepressant drug fluoxetine has been found to inhibit TREK-1 channels [16].

Another major problem is that most of the ion channels claimed to promote cancer are also expressed and have a clear function in many other non-affected tissues. Local application of the channel inhibitors or tissue targeting of the drug may circumvent systemic effects. Another essential question is, how much of the ion conductance must be inhibited to interfere with proliferation of the cancer? Would it be even more effective to activate K^+ or Cl^- conductances in order to induce cell shrinkage and apoptosis? These and many more questions need to be answered before we may start with an ion channel therapy for cancer. A major goal is to identify new potent drugs that selectively inhibit or activate K^+ or Cl^- or Ca^{2+} channels overexpressed in tumor tissues [24]. Moreover, we need more studies on native cancer tissues and good animal models to examine the function of the oncogenic ion channels in the ''real'' tissue. Finally, studies are missing that correlate the staging of the tumor with the appearance of the ion channel. It is currently not clear whether oncogenic ion channels are expressed at an early stage of the mutated cell or whether these channels are expressed only at a late stage of the cancer, as suggested for some carcinoma [12]. By putting more effort into this research it should be possible in the near future to attack this deadly disease by manipulating ion channel activity.

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