Voltage-Gated Potassium Channels in Cell Proliferation

It is commonly accepted that cells require K⁺ channels to proliferate. The role(s) of K⁺ channels in the process is, however, poorly understood. Cloning of K⁺ channel genes opened the possibility to approach this problem in a way more independent from pharmacological tools. Recent work shows that several identified K⁺ channels are important in both physiological and pathological cell proliferation and open a promising pathway for novel targeted therapies.

As a crucial cellular function, cell proliferation is very strictly controlled by several independent mechanisms. Evidence has accumulated pointing to K⁺ channels as relevant players in the control of this process since the pioneering work in lymphocytes by DeCourcy et al. (26). However, like in any complex physiological process, establishing an unequivocal link between a simple molecular event and physiological or pathological cell proliferation requires a great extent of inductive arguments. The present review will attempt to summarize these arguments, centering in those (few) cases in which the association has been established on more solid basis.

In general, inhibition of K⁺ channel function leads to a decrease in proliferation both in models in which proliferation is a physiological response (the case of lymphocytes) and in those in which it is a manifestation of a pathological condition (as in cancer cells). In 1996, Wonderlin and Strobl published an exhaustive review (104) that has become a mandatory reference in the field; in that paper, they provide a thorough list of cell lines in which nonspecific K⁺ channel blockers such as 4-aminopyridine, quinidine, and TEA have antiproliferative effects: lymphocytes, brown fat cells, Schwann cells, melanoma, breast carcinoma, neuroblastoma, small-cell lung cancer, and bladder cancer cells. The list of models has grown since that publication (e.g., neuroendocrine cells (46), hepatocytes (54), GH3 pituitary cells (95), endothelial cells (29), keratinocytes (61, 102) corneal epitheli- um (78), retinal pigment cells (38), chondrocytes (101), myeloblastic leukemia (97, 106, 107), prostate cancer (89), hepatocarcinoma (108), mesothelioma (94), colon cancer cells (1, 51), but the bottom-line message remains the same: if K⁺ channels are inhibited, proliferation is impaired. The obvious conclusion is that cells need K⁺ channels to proceed with the cell cycle. More specifically, initiation of G₁ progression requires in general K⁺ channel activity (33, 84, 104).

Pharmacological approaches often do not give deep insight into the molecular mechanisms underlying such effects. Many K⁺ channel inhibitors require high doses to show effects on proliferation, and many (if not all) have effects on totally unrelated proteins at such high concentrations; nonspecific effects could therefore be argued in many cases. Additionally, even if we accept that the effects of such agents are specific on K⁺ channels, there are very few available specific inhibitors for a certain K⁺ channel type, with the exception of some toxins. The identification of the specific K⁺ channel(s) implicated in the control of the proliferation of a given cell type is therefore difficult.

In any case, it is intuitively acceptable that K⁺ channels, as key players in controlling membrane potential, are important in proliferation processes. It has been known for a relatively long time that cancer cells are on average more depolarized than nontumor cells (4), although a transient hyperpolarization is required for the progression of the early G₁ phase of the cell cycle (104). Thus blockade of K⁺ flux, which leads to depolarization, should interfere with proliferation by inhibition of such transient hyperpolarization.

The effect of membrane potential variations under the control of K⁺ channels would not only be the regulation of Ca²⁺ influx (67), which is well established as a crucial factor for cell proliferation, but also maintaining the driving force for Na⁺-dependent nutrient transport and influencing the intracellular pH. The only requirement for a particular K⁺ channel to perform this hyperpolarizing function would be to have the adequate threshold potential of activation. Still, is there just a quantitative effect on membrane potential that any K⁺ channel (with the right activation threshold) can achieve, or is there a much more complex modulatory activity that only certain channels at specific times or locations can perform? If just the effects on membrane potential would be sufficient to alter the cell cycle progression, that is, if blocking any K⁺ channel impairs proliferation, the opposite effect should also be expected. Thus cycle progression would also be affected (accelerated) by an increase in the expression levels of endogenous (or intro-
duction of exogenous K+ channels. If this were true, K+ channel openers should be oncogenic (and fortunately they do not seem to be so, although in some preparations K+ channel openers have been reported to increase DNA synthesis (37, 60), and introduction of K+ channels under the control of strong promoters should also induce transformation. Although most (if not all) cloned K+ channels have been expressed in heterologous systems under strong promoters, only for Kv10.1 (71) has transforming activity been observed using another K+ channel (Kv1.4) as negative control. Therefore, it is probably more logical to assume that the specific type of K+ channel is important for proliferation-related processes.

K+ channels are among the most diverse families of membrane proteins. The IUPHAR compendium of voltage-gated ion channels (16) mentions 75 different genes for K+ channels, as opposed to 9 or 10 different Na+ or Ca2+ channel genes. Such diversity gives enough possibilities to generate K+ channels with the exact properties required for cell proliferation. Additionally, the temporal and spatial distribution of K+ channels, as well as the formation of supramolecular structures, is probably relevant to the function of channels during the cell cycle. With respect to association with other proteins, it has been shown that several K+ channels physically interact with important effectors in proliferation-related processes, like Kv11.1 with 14-3-3 (44), Src (17), or TNF-α receptor (96), Kv1.3 with β-integrins (53) and p56lck (36, 92) or Kv10.1 with calmodulin (86).

If the only action of K+ channels were directly related to potential changes, K+ channel blockers would need to induce depolarization of the cells to inhibit proliferation. In a similar way, it would be expected that the blockers also affect intracellular Ca2+ levels. However, neither prediction is true in all cases (e.g., Refs. 60 and 81), indicating a more complex mechanism. Such discrepancies are the basis for an alternative hypothesis that postulates that K+ channels would be important for proliferation because they influence cell volume (79). K+ channels, in combination with Cl- channels, are necessary for regulatory volume changes. Interestingly, Cl- channels have also been associated with proliferation in many cell types (e.g., Refs. 50, 88, and 103). Proliferation is associated with volume increase along the G1 phase, but nonspecific cell swelling can inhibit proliferation (50), indicating a direct (probably biphasic) link between both processes. In fact, glioma cells show their highest proliferation rate within a relatively narrow range of cell volumes, with decreased proliferation both over and under that optimal range (80). Although the existence of a “cell size checkpoint” in mammalian cells similar to the one described for lower eukaryotes is dubious (23), the cell volume needs to be controlled in such a way that crucial solutes [and cell cycle-regulating proteins themselves (80)] maintain a concentration appropriate to support proliferation.

Recently, Kv1.3 knockout mice were produced and analyzed (48). As we will describe below, Kv1.3 has been shown to play a crucial role in T lymphocyte physiology, but the knockout mice show a normal immunological activity (48). This is apparently achieved by a compensatory, up to 50-fold, increase on Cl- channels. Cl- channels can compensate for an alteration of the membrane potential, but this would have opposite effects to those of K+ channels on volume regulation, although other concomitant compensatory mechanisms cannot be excluded.

Certainly both changes in membrane potential and cell volume are necessary for progression of the cell cycle (FIGURE 1), and both require the action of K+ channels. If the particular channels implicated in both processes were not the same, it would be conceivable that some drugs would interfere with proliferation through volume regulation and some others by interference with membrane potential changes.

Pharmacological tools opened the way toward understanding of the role of K+ channels in cell proliferation but are limited because of their lack of specificity when the identification of the individual K+ channels was attempted. More direct evidence regarding the role of K+ channels on proliferation was possible after the molecular entities underlying the K+ currents were cloned. Yet until now only a few types of K+ channels have been identified to correlate with cell proliferation.

**FIGURE 1. Hypothetical relations between K+ channels and cell proliferation**

Mitogenic signals reach signaling supramolecular complexes that transfer the signal to the K+ channel either because the channel itself is a physical part of the complex or indirectly through second messengers. The exact nature of the effect of channel activation will depend on the particular entity activated and will result from a combination of effects related to membrane potential changes and cell volume regulation.
The cases best studied correspond to Kv1.3 (KCNA3) and IKCa1 (KCNA3, KCNN4), which govern the mitogenic response in lymphocytes, but also HERG (Kv11.1, KCNHI2), Eag1 (Kv10.1, KCNH1), and TASK-3 (KCNN9, KCNN9). The latter are expressed in tumor cells and have shown a clear correlation to cell growth. Since such a large number of cell types show dependence on K⁺ channels for proliferation, these are probably just the first examples of a growing group of K⁺ channels involved in cell proliferation.

In an attempt to avoid nomenclature misunderstandings (especially frequent for K⁺ channels) all channels will be termed using the IUPHAR convention (16) from now on. Table 1 summarizes the nomenclature equivalences for those channels mentioned here.

Kv1.3/ KCa3.1

Although Kv3.1 is not a voltage-gated K⁺ channel, its physiological role in lymphocyte proliferation is conceptually very difficult to separate from that of Kv1.3, and we will refer to them together. As already mentioned, this was the first case where a clear link between K⁺ channels and mitogenesis was established (26), and a number of excellent and exhaustive literature reviews are available (e.g., Refs. 14 and 18). Kv1.3 (91) is a typical member of the 6TM1P [six transmembrane segments (TM) with one pore loop between the fifth and sixth TM] structural group. It gives rise to a noninactivating, delayed-rectifier current showing cumulative inactivation. Kv3.1 (39, 42, 55) is a Ca²⁺-dependent K⁺ channel. It appears to be constitutively associated to calmodulin (even in the absence of Ca²⁺), and binding of Ca²⁺ to this associated calmodulin induces a conformational change in the channel that opens the permeation pathway (41).

It has been demonstrated that K⁺ channel activity is required for migration, adhesion, and volume regulation in T cells (14), but the best-studied process requiring K⁺ channels is activation (3, 26, 35, 52). The term activation refers to the process by which immune cells react to the presence of an antigen and renders cells ready to proliferate to produce an effective immune response. This activation is inhibited by blocking K⁺ channels (26). This phenomenon is physiologically relevant, for example, in the process of immunotolerance at the placenta. Progesterone inhibits Kv1.3 and Kv3.1 channels, and the activation of T cells is impaired where the levels of the hormone are high (at the placenta), whereas the activation remains normal in the rest of the mother’s organism (28). In a similar way, Kv1.3 modulation by hypoxia might be the reason for the immunosuppressive effects of low PO₂ (22).

Table 1. Potassium channel nomenclature

<table>
<thead>
<tr>
<th>IUPHAR Name</th>
<th>Locus</th>
<th>Other Common Name(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kv1.3</td>
<td>KCNA3</td>
<td>MK3, RCK3, Kv3, n channel</td>
</tr>
<tr>
<td>Kv10.1</td>
<td>KCNH1</td>
<td>eag1, ether-à-go-go</td>
</tr>
<tr>
<td>Kv11.1</td>
<td>KCNH2</td>
<td>HERG, erg</td>
</tr>
<tr>
<td>KCa3.1</td>
<td>KCNN4</td>
<td>SKA4, IKCa1, Gardos channel</td>
</tr>
<tr>
<td>KCa3.1</td>
<td>KCNK9</td>
<td>KCNK9, TASK-3</td>
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Kv1.3 is the dominant channel in resting T cells (27). Activation results in a dramatic increase in Kv3.1 channels that start to dominate the picture (together with an increase in Ca²⁺ release-activated current channels) (14). Since the channel phenotype switches during the process of activation, a selective blockade of Kv1.3 channels prevents activation without affecting the response of preactivated cells, whereas these are inhibited by specific KCa3.1 blockers (49). Under pathological conditions, like multiple sclerosis (MS), it has been shown that activated lymphocytes retain a "preactivated" phenotype (high Kv1.3, low KCa3.1). In animal models for MS, as well as in lymphocytes from MS patients, the density of Kv1.3 channels is severalfold higher than in normal resting cells, as opposed to normal activated T cells (7, 8). This might underlie the memory phenotype of MS-activated lymphocytes while opening a possible therapeutic window, since these cells are sensitive to anti-Kv1.3 therapies, whereas blockade of Kv1.3 can be overcome by normal T lymphocytes because they upregulate Kv3.1 (105).

Besides the implication in physiological T lymphocyte activation, Kv1.3 has also been reported to be involved in proliferation of other cell types, such as oligodendrocyte progenitor cells (21), glioma (75), and to different extents in prostate cancer (1, 32), colon cancer (1), breast cancer (2), and melanoma cells (6). Similarly, Kv3.1 appears to be involved in the control of proliferation of at least some cancer cell lines (72).

KcnB2 9.1

KcnB2 9.1 (TASK-3, KCNK9) (19, 47, 77) is a member of the 4TM2P K⁺ channel family: that is, it probably possesses four transmembrane segments and two pore domains per subunit (as opposed to 6TM1P of the "classical" K⁺ channels). The complete channel is probably a dimer. Like other 4TM1P channels, it most likely carries an "instantaneous" background K⁺ current that helps set the resting membrane potential and is highly sensitive to extracellular pH. A functional interaction of the channel with the signaling adaptor protein 14-3-3 has been described (76).

Expression analysis of cancer samples revealed...
that the KCNK9 gene was amplified in 24 out of 64 breast cancers and with varying frequencies in other tumor types (65). K_9.1 overexpression also enhanced the tumorigenicity of cells in subcutaneous implantation in nude mice as well as the resistance to conditions like low serum or low P_O2 in the culture. However, it did not induce malignant transformation on its own (65). The ability of the channel to produce its tumor-enhancing effects requires its function as a K^+ channel, since a point mutation abolishing its ability to permeate potassium also eliminates its oncogenic capabili-
ties (73).

**Kv10.1 and Kv11.1**

The *ether-à-go-go (eag)* channel family was initially described in *Drosophila melanogaster* during mutagenesis experiments (45). Positional cloning allowed the identification of the gene responsible for the mutated phenotype, and it soon became obvious that the gene had remarkable structural homology with K^+ channels (98), as later confirmed by its functional expression analysis (11). The search for mammalian homologs of *eag* allowed the identification of three mammalian subfamilies: *eag* (Kv10), *erg* (Kv11), and *elk* (Kv12) (99). Two of the channels, Kv10.1 and Kv11.1, have been implicated in proliferation processes, mainly in tumor proliferation.

The primary structure of these channels corresponds to the 6TM1P group. Like in the case of Kv channels, the functional EAGs are tetramers, formed by either identical subunits or subunits encoded by genes from the same subfamily. That is, Kv10.1 and Kv10.2 are able to form heteromultimers, and so are Kv11.1, 2, and 3 (100), but Kv10 and Kv11 do not interact (40). The molecular determinants for tetramerization are located at the distal COOH-terminal end of the channels (57) and consist of a coiled-coil structure with a very strong tendency to tetramerize (40). Despite structural similarities, there are striking functional differences between EAG channels and other 6TM1P K^+ channels.

Kv11.1 (Human Eag-related gene, KvH9, KCNH2) was first identified during screening for a human *eag* by homology to the *Drosophila* gene (99). Soon thereafter, it deserved wide attention due to its identification as molecular substrate of some forms of familiar and acquired long QT syndrome (25, 83). For this reason, there is abundant literature on Kv11.1 in the heart and considerable knowledge about relevant structural features of the channel. Kv11.1 shows very fast inactivation kinetics that result in significant current through the channel passing only after repolarization of the membrane potential (93). Three different genes constitute the Kv11 subfamily in mammals (Kv11.1, Kv11.2, and Kv11.3 (39)). Kv11.2 and Kv11.3 are expressed only in nervous tissue. Due to its peculiar kinetics, Kv11.1 shows maximal open probability at values close to the typically depolarized resting potential of tumor cells. The initial evidence linking Kv11.1 to proliferation was obtained through the observation at Enzo Wanke’s laboratory that in cycling cells, like neuroblastoma, the voltage dependence of activation of Kv11.1 varies during the cell cycle, in good correlation with the resting potential variations (4, 5, 30). This led to the conclusion that Kv11.1 could govern the resting potential of these cells, thus influencing their proliferation. Following this lead, Kv11.1 expression was also found to be upregulated in several tumor lines from different histogenesis (9), compared with the corresponding normal cells. Kv11.1 expression would offer the cancer cells a selective advantage for proliferation, since it can contribute to the maintenance of a depolarized potential. In the tumor environment, extracellular K^+ is typically elevated and P_O2 is reduced, conditions that could contribute to higher Kv11.1 activity, since elevated extracellular K^+ increases the open probability of the channel and hypoxia chronically modulates Kv11.1 (31). Additional mechanisms pointing to the participation of Kv11.1 in the regulation of cell proliferation are indicated by its interaction with proteins implicated in mitogenesis. Kv11.1 interacts with Src tyrosine kinase (17) and with signaling adaptor proteins of the 14-3-3 type (44). Recent evidence shows that alternatively spliced Kv11.1 variants are differentially expressed during the cell cycle (24). Kv11.1a (HERG1A) is the “standard” form of the channel, but it coexists with a shorter (NH_2-terminal-truncated) isoform, Kv11.1b (HERG1B). Both isoforms can coassemble into heteromultimers. Kv11.1b shows faster activation kinetics (56) that could explain the different kinetics observed in neuroblastoma cells during the cell cycle, since this isoform is mainly expressed during the S phase of the cell cycle. It has been postulated that hypoxia is the trigger that switches the channel expression between both forms (24), since Kv11.1b lacks the PAS domain common to all eag channels that could serve as a P_O2 sensor (13). Although the exact meaning of alternative splicing of Kv11.1 remains obscure, the fact that not only the protein levels but also the defined molecular composition of the channel may vary during the cell cycle is probably an indication of its relevance for proliferation. Indeed, it has been shown that inhibition of Kv11.1 currents in several cell lines leads to a reduction in tumor cell proliferation (24, 90). Cell lines of tumor origin often show profound phenotypical and genotypical alterations due to
their maintenance in culture. For this reason, expression of a particular protein in tumor cell lines is usually considered an indication but no proof of their expression in primary tumors. The limited availability of primary tumor cells makes it more difficult to collect the data. To date, Kv11.1 expression has been shown in primary leukemias (74), endometrial tumors (20), and colon cancer (51).

Kv11.1 also appears to influence other related processes, like apoptosis. It has been shown that cells expressing Kv11.1 are more sensitive to apoptosis induced by H2O2 than those that do not and that blockade of Kv11.1 by dofetilide reverts this situation (96). The same authors (96) showed that there is physical interaction between the channel and TNF-α receptor 1 in the membrane of tumor cells.

Two different orthologs of the Drosophila eag gene have been identified in mammals: Kv10.1 (Eag1, KCNH1) (36, 68) and Kv10.2 (Eag2, KCNH5) (43, 59, 82). Both give rise to slowly activating currents with a marked dependence on extracellular divalent cations and prepulse potential (e.g., Ref. 85). Only Kv10.1 has to date been implicated in proliferation processes.

The first indications of a possible role in the cell cycle came from the observation that in Xenopus oocytes (12) the functional properties of the channel change dramatically when the cell cycle progresses. Oocytes offer a convenient model for the study of the cell cycle, because they are physiologically arrested in the G2 phase of the first meiotic division and the progression of the meiosis can be induced by extracellular application of progesterone. This induces the activation of mitosis-promoting factor (MPF), which is the protein kinase responsible for the initiation of mitosis in all tested cell types. The kinetic change in Kv10.1 function consists of a dramatic rectification; that is, the current becomes smaller (instead of larger) the more positive the membrane potential is set. The change in kinetics is a reflection of a change in permeation properties, due to a blockade by intracellular sodium (70), and is likely to be induced by alteration of the interactions between the channels and the cytoskeleton (15).

In Northern blot analysis, Kv10.1 is expressed in the central nervous system at high levels, but it could not be detected in other tissues, except for placenta (68, 71) (expression in the placenta has not been confirmed by other techniques). Kv10.1 expression has also been shown in myoblasts, although it is restricted to a narrow temporal window at the onset of fusion (10). In our group, we had detected expression of Kv10.1 in breast carcinoma MCF7 cells, both functionally and at the level of RNA, but we could also show that the channel is not expressed in normal human mammary gland epithelial cells (71). The expression of Kv10.1 can also be detected in several other tumor cells (62, 63). In tumor cell lines, Kv10.1 probably confers a selective advantage for tumor progression. Transfection of Kv10.1 into cells that normally do not express it induces a transformed phenotype: the cells grow faster, are able to grow in the presence of low levels of growth factors and in the absence of substrate attachment, and lose contact inhibition (71). These properties classically define oncogenes. Inhibition of the expression of Kv10.1 by antisense technology (71) or pharmacological means (34, 69) leads to a reduction in proliferation in cell lines expressing this channel. Finally, Kv10.1 expression favors the progression of experimental tumors in scid mice (71). In neuroblastoma cells, Von-Hippel-Lindau (VHL) expression induces differentiation into a neuronal phenotype and a concomitant Kv10.1 downregulation (66). VHL is a protein implicated in the response to hypoxia, and this finding might indicate a relationship between hypoxia (common in tumors) and Kv10.1 expression. It is interesting to point out that hypoxia is the only stimulus that has been identified as a direct or indirect modulator of Kv1.3, Kv10.1, Kv11.1, and K2P9.1.

Conclusions

In summary, several K⁺ channels (identified at the molecular level) have been shown to be directly implicated in normal and/or pathological cell proliferation. This has obvious medical consequences that are just starting to be explored. Inhibition of Kv1.3 and/or K⁺3.1 interferes with immune cell activation and has been shown to have immunosuppressive effects (87). A particularly promising field of application are autoimmune diseases, and more specifically MS (7, 8, 105), since the cells responsible for the pathological condition show an altered channel pattern that would allow leaving the normal cells relatively unaffected. Moreover, it has been possible to isolate compounds that are able to moderately distinguish between Kv1.3 from brain and from T lymphocytes (64), opening the possibility for a highly selective therapeutic application on immune cells.

K2P9.1, Kv11.1, and Kv10.1 are overexpressed in tumors and are therefore candidate targets for anticancer therapies. Since these membrane proteins are extracellularly accessible, they are all good candidates for immunotherapeutic approaches but also for classical small-molecular-weight pharmaceutical agents. Inhibition of Kv10.1 and Kv11.1 has been shown to reduce the proliferation rates of tumor cell lines (24, 34, 69). K⁺9.1 was the last channel to be identified in tumor cells, and little is
known about its pharmacology to date, but this is certainly an example of the growing number of channels involved in cell proliferation and cancer. The pharmacology of Kv11.1 has been studied in depth, and many inhibitors are available. The main issue with a potential therapy targeted against Kv11.1 is the necessity to avoid potentially lethal cardiotoxicity induced by interference with the cardiac Kv11.1. A drug selectively blocking the truncated isoform Kv11.1b, which is enriched in tumor cells but absent from the adult heart, could overcome this problem (24). Kv11.1 might offer the simplest approach, due to its very restricted expression pattern. A drug effectively excluded at the blood-brain barrier would not affect Kv10.1 channels filling their normal physiological functions. In any case, it is predictable that one of more of these molecular entities will become a relevant anti-cancer target in the coming years.

I thank Walter Stühmer for his continued support over the years and for critically reading this manuscript, as well as all the members of his department and especially of the EAG group.

A complete list of references would be impossible to fit in the limited space. I apologize to all scientists that made relevant contributions in this field and were not given adequate credit in this review.

The author is a shareholder (and served as CSO between 2001 and 2003) of iOnGen, a company developing ion channel-targeted therapies against cancer.

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