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# Clinical Relevance of Ion Channels for Diagnosis and Therapy of Cancer

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**Abstract.** Ion channels have a critical role in cell proliferation and it is well documented that channel blockers can inhibit the growth of cancer cells. The concept of ion channels as therapeutic targets or prognostic biomarkers attracts increasing interest, but the lack of potent and selective channel modulators has hampered a critical verification for many years. Today, the knowledge of human ion channel genes is almost complete and molecular correlates for many native currents have already been identified. This information triggered a wave of experimental results, identifying individual ion channels with relevance for specific cancer types. The current pattern of cancer-related ion channels is not arbitrary, but can be reduced to few members from each ion channel family. This review aims to provide an overview of the molecularly identified ion channels that might be relevant for the most common human cancer types. Possible applications of these candidates for a targeted cancer therapy or for clinical diagnosis are discussed.

**Key words:** Ion channels — Cancer — Therapeutic target — Prognostic marker

## Introduction

Ion channels are well known for their roles in a large variety of physiological functions, including electrical signaling, control of muscle contraction, volume regulation, and hormone secretion. In addition to these beneficial activities, channel proteins have been proposed to promote the complex process of tumor formation by various mechanisms. This pathophysiological function is one of most interesting new research areas for scientists working on ion channels and promises new therapeutic strategies in the fight against cancer. The increased interest becomes evident by a growing number of publications, describing the mere occurrence of individual ion channels in tumor cells, or the functional consequences of ion channel blockade on cellular functions like growth, migration, or invasion. But does the clinical oncologist already recognize ion channels as potential new tools in his daily routine? Until today, ion channels are neither on the list of routinely assayed prognostic factors for any cancer type, nor did channel antagonists or agonists reach the clinics as therapeutic agents for cancer treatment. The current state may be illustrated by a look at the proceedings of two major oncology meetings. While 115 presentations (from a total of 6184) at the 96th annual meeting of the American Association for Cancer Research (AACR) were dealing with ion channels in any form, no contribution at the more clinically-oriented meeting of the American Society of Clinical Oncology (ASCO) was dedicated to channels. Apparently, the recognition of ion channels as cancer-related factors depends on the individual point of view and might range somewhere between "hot topic" and "peculiar anecdote". The present review is intended to highlight ion channels that may soon become therapeutic targets or prognostic markers for the most common cancers. While this list will not be complete, it may open the stage for a closer evaluation of channels, blocking agents, and diagnostic applications as candidates for clinical use in the near future.

## Which Tumors and which Channels?

The last two decades brought a remarkable increase of published evidence for the role of ionic currents and individual ion channels in tumor progression.

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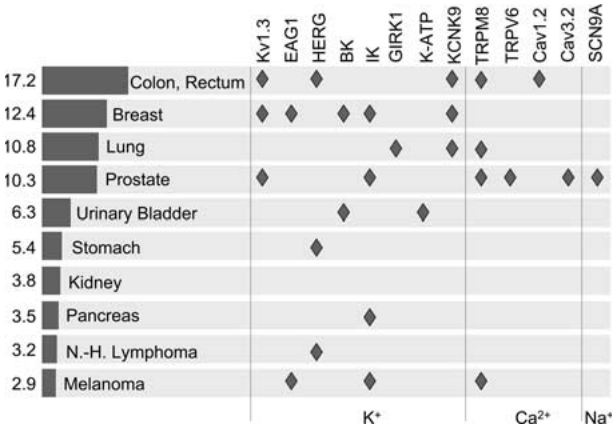


**Fig. 1.** Common cancers and ion channels. Bar graphs on the left represent incidence rates (in %) for the ten most common cancer types in Germany (Bertz et al., 2004). Gender-specific differences are not reflected. For women, breast cancer shows the highest incidence rate (24.4 %) while prostate cancer is the most prominent cancer type in men (20.3 %). On the right, up-regulated ion channels with potential relevance for the respective cancer type are indicated. Ion symbols refer to the respective selectivities of the listed ion channels. Note that TRPM8 is $Ca^{2+}$-permeable but not selective.

Reviews on this topic are usually focused on channel classes, such as potassium channels or chloride channels, or on smaller channel families, and even individual proteins. This is a very useful approach for an identification of structure-function relations or potential disease mechanisms. However, in order to make assumptions about a clinical relevance of ion channels in cancer, it may be informative to change direction, and to ask which channels are found in a given tumor type? Are there specific patterns, such as cancer types with a high prevalence of ion channels and others without recognizable ion channel profile? In an attempt to address these questions, the top ten list of cancer incidences will be discussed with respect to ion channels that have been identified in these cancers and that are under suspicion to affect the progression of tumors in any form (Fig. 1). The given numbers for cancer incidences or death rates are based on German cancer statistics (Bertz et al., 2004) or on estimates for the United States, as published by the American Cancer Society (Jemal et al., 2005).

Cancer of colon and rectum the most common cancer type in Germany and the second leading cause of cancer deaths. As depicted in Fig. 1, at least five different ion channels with aberrant expression have been identified in colorectal cancer cells. An α-subunit of L-type voltage-gated calcium channels (Cav1.2) showed strongly increased mRNA levels in colon cancer biopsies in comparison to normal colonic mucosa (Wang et al., 2000). This channel type is almost ubiquitously expressed in excitable cells, but rather unexpected in colon mucosa (Catterall, 2000). mRNA of another $Ca^{2+}$-conducting channel (TRPM8, also called Trp-p8) was shown to be expressed in colorectal adenocarcinoma, but not in healthy colon (Tsavaler et al., 2001). TRPM8 is a member of the more recently discovered transient receptor potential (TRP) family (Clapham et al., 2003). While many of these channels are $Ca^{2+}$-permeable, they are structurally more closely related to voltage-gated potassium channels with typically six transmembrane-spanning segments per subunit. While TRPM8 has a physiological function as cold sensor, a potential activation mechanism in tumor cells is unknown. At least three different potassium channels were detected in colon cancer cells. Kv1.3, a typical voltage-gated potassium channel, was detected by immunohistochemistry in 74 analyzed colonic carcinoma specimens, while two normal colon tissue samples did not contain detectable Kv1.3 channels (Abdul & Hoosein, 2002a). More recently, the voltage-gated HERG potassium channel was detected in colon cancer cells (Lastraioli et al., 2004). Immunohistochemical analysis of biopsy material revealed no HERG protein in non-cancerous colonic mucosa but high expression in metastatic colon carcinoma samples (Lastraioli et al., 2004). HERG mRNA and protein were also found highly expressed in colon cell lines, and channel activity was required for an invasive phenotype of these cells in vitro (Lastraioli et al., 2004). In an immunohistochemical survey of 124 colorectal cancers, about half of the samples were immunopositive for the KCNK9 potassium channel (Kim et al., 2004). KCNK9 belongs to a family of leak channels without intrinsic voltage dependence (Patel & Lazdunski, 2004). The normal expression of KCNK9 is highly restricted to the brain and in particular to the cerebellum (Chapman et al., 2000; Rajan et al., 2000). In contrast to the given examples of up-regulated ion channels, two calcium-activated chloride channels (CLCA1 and CLCA2) were considered as potential tumor suppressors. Transcriptional down-regulation of both genes was detectable in over 80% of the tested colorectal tumor samples (Bustin et al., 2001).

Breast cancer has by far the highest incidence rates of cancer in females, both in Germany (24%) and the USA (32%). Many different ion channels have been attributed to breast cancer cells and one of the best-described breast carcinoma cell lines is MCF-7. In 1999, Pardo and coworkers detected mRNA of the voltage-gated EAG1 potassium channel in MCF-7 cells, but not in the unaffected mammary gland (Pardo, et al., 1999). Ouadid-Ahidouch recorded typical EAG1 currents from this cell line and found them to be regulated by the cell cycle (Ouadid-Ahidouch et al., 2001). Functional expression in MCF-7 was also reported for calcium-activated potassium channels of intermediate conductance (IK) and large conductance (BK; Ouadid-Ahidouch et al., 2004a; Ouadid-Ahidouch et al., 2004b). In a survey of 60 human breast cancer specimens, Abdul and coworkers found all samples immunopositive for the

voltage-gated Kv1.3 channel, while normal human breast was negative (Abdul, Santo & Hoosein, 2003). Kv1.3 and the calcium-activated IK channel are normally expressed in T-lymphocytes, and both function as critical regulators of T-cell proliferation after immunostimulation (Chandy et al., 2004). The reports listed so far were mostly aimed to identify channel activity in cancer cells. In contrast, overexpression of KCNK9 potassium channels in breast cancer was revealed by a different approach. Mu and coworkers analyzed chromosomal amplifications in breast tumors (Mu et al., 2003). This analysis identified an amplicon containing KCNK9 as the only overexpressed gene. Genomic amplification was found in 24 out of 247 primary breast tumors (10%), but increased mRNA levels of KCNK9, independent of a genomic amplification, were observed in 44% of the analyzed samples (Mu et al., 2003). A functional relevance of KCNK9 channels in breast cancer cells was suggested, as overexpression of the channel in cell lines promoted tumor formation in a mouse model (Mu et al., 2003). In close analogy to colon cancer, an inverse correlation of chloride channel CLCA2 expression and tumorigenicity was also described for breast cancer (Gruber & Pauli, 1999; Elble & Pauli, 2001). Stable transfection of the CLCA2 chloride channel gene into tumorigenic breast cancer cell lines resulted in reduced invasion in vitro and lower metastatic potential in nude mice (Gruber & Pauli, 1999). Interestingly, CLCA2 channel proteins are also involved in the mediation of breast cancer metastases in the lung. However, in this case, CLCA2 is expressed on endothelial cells in pulmonary arteries, and was identified to form an adhesion anchor for blood-borne breast cancer cells, which bind via beta 4 integrin molecules on the surface of the cancer cells (Abdel-Ghany et al., 2001).

Cancer of the lung and bronchus system is among the three most commonly diagnosed cancers and accounts for a cancer death rate of almost 20% in Germany and over 25% in the United States. Only less than 20% of lung cancers are diagnosed at an early, localized stage and in contrast to many other cancer types, including colon and breast cancer, even early detection cannot ensure a five-year survival rate of more than 50%. While new therapeutic targets for this cancer are urgently needed, reports of ion channels involved in lung cancer progression are sparse. In situ hybridization with a TRPM8-specific RNA probe revealed expression of this calcium-conducting channel in eight out of ten tested samples encompassing various lung cancer types (Tsavaler et al., 2001), but functional expression has not yet been investigated. The KCNK9 potassium channel was also detected at the level of mRNA (Mu et al., 2003). In five of ten tumor samples the mRNA of KCNK9 was at least 10-fold up-regulated (Mu et al., 2003). An additional potassium channel, attributed to lung cancer, belongs to the family of G protein-coupled inwardly rectifying potassium channels (GIRK). From a group of 72 non-small-cell lung cancer patients 50 samples exhibited strong GIRK1 expression, and the presence of this mRNA correlated significantly with reduced five-year survival rates (Takanami et al., 2004).

Prostate cancer has the highest male incidence rate (20.3%) in Germany and incidences are even higher in the United States (33%). These numbers may correlate in part with the intensity of screening efforts for prostate-specific antigen (PSA). Unfortunately, PSA levels alone are insufficient to guide the decision whether radical treatment is required for an individual patient or not. By screening a prostate-specific subtracted cDNA library, Tsavaler and coworkers identified the TRPM8 channel protein as a prostate-specific marker being up-regulated in tumor tissue. (Tsavaler et al., 2001). The same gene was independently identified as highly prostate-specific in a genome-wide search for prognostic targets using expression profiles (Henshall et al., 2003). Interestingly, this report also showed that elevated TRPM8 levels of tumor samples are again decreasing with tumor progression to an androgen-insensitive late stage. According to these data, loss of TRPM8 is a significant marker of a poor prognosis. A second member of the TRP channel family with strong expression in prostate cancer is the highly $Ca^{2+}$-selective TRPV6 (also called CaT-L) (Wissenbach et al., 2001; Peng et al., 2001). The channel was not detected in healthy prostate tissue and benign prostatic hyperplasia (Wissenbach et al., 2001). In situ hybridization on 140 prostate tissue specimens revealed significant correlation to the Gleason score and the pathological stage (Fixemer et al., 2003). The voltage-gated potassium channel Kv1.3 might also be relevant in prostate cancer. Strong immunostaining was detected in 37 of 79 prostate cancer samples (Abdul & Hoosein, 2002b) and electrophysiological data, combined with RT-PCR and Western blots, confirmed the presence of Kv1.3 channels in rat prostate cancer cell lines (Fraser et al., 2003). There is also electrophysiological evidence for a prominent expression of calcium-activated IK channels in rat prostate cancer cells (Rane, 2000). The functional impact of IK potassium channels in prostate cancer was supported by pharmacological intervention experiments, using the human cell lines LNCaP and PC-3 (Parihar et al., 2003). 1-EBIO, a known activator of IK and related potassium channels, triggered a concentration-dependent increase of proliferation in both cell lines, while the more specific inhibitors clotrimazole and charybdotoxin prevented this effect (Parihar et al., 2003). In addition to potassium channels, several types of voltage-gated sodium channels have been distinguished in prostate cancer cell lines (Lanaido et al., 1997; Diss et al., 2001).

Comparison of cell lines with differing metastatic potential suggested a role of these sodium channels, and specifically of SCN9A (also termed PN1 or Nav1.7) in invasive migration (Diss et al., 2001). The specific block of voltage-gated sodium channel activity in the metastatic cell line MAT-LyLu by tetrodotoxin was found to decrease the cell motility without affecting the cellular proliferation rate (Fraser et al., 2003). Just as voltage-gated sodium channels, voltage-gated calcium channels are classically associated with functions in excitable cells rather than in non-excitable epithelial cells. However, Mariot and colleagues observed a strong upregulation of T-type calcium channels (Cav3.2) in LNCaP cells during neuroendocrine differentiation, a process associated with increased invasiveness and poorer prognosis (Mariot et al., 2002).

Cancers of the urinary bladder account for about 6% of cancer incidences and 3% of cancer-related death rates, but the presence of ion channels in bladder cancer and their potential contribution to the malignant phenotype are only poorly examined. In a search for potassium channels in the bladder carcinoma cell line HTB-9, Monen and coworkers recorded potassium currents with properties and pharmacological profile of large-conductance calcium-activated channels (BK) and of ATP-sensitive potassium channels (K-ATP; Monen et al., 1998). Addition of glibenclamide to the culture medium, at concentrations sufficient to block the ATP-sensitive current, resulted in a significant growth inhibition of this cell line, implying a role of K-ATP channels in cell proliferation (Wondergem et al., 1998).

In Germany, cancer of the stomach accounts for roughly 5% of cancer incidences and cancer-related deaths, while both numbers are below 3% in the United States. Like with many other cancer types, only sparse information is available on ion channels in gastric cancer cells. A recent survey for HERG potassium channels revealed a clear cancer-limited expression, both in cell lines and tumor tissue (Shao et al., 2002). Inhibition of HERG channels by the known channel blocker cisapride inhibited the cellular proliferation of gastric cancer cells in a dose-dependent fashion and resulted in cell-cycle arrest at the G(1) to S phase transition (Shao et al., 2002).

Cancers of the kidney and renal pelvis have incidence and death rates around 4% but they typically have a poor prognosis, as early detection is difficult. Also renal cancers are not on the list of tumor entities known for a high prevalence of ion channels. A preliminary screen in our laboratory for voltage-gated or calcium-activated potassium currents in renal clear cell carcinoma lines did not reveal significant functional expression of these channel types (Niedling, *unpublished*).

Pancreas cancer has an incidence rate of 3.5% in Germany, but its contribution to cancer deaths is around 6%. The extremely poor prognosis for this cancer results in part from the fact that early detection at a localized stage is rare. But even if a localized tumor is diagnosed, the five-year relative survival rates are below 20% and new therapeutic strategies are certainly required. There is convincing evidence for a functional expression of intermediate-conductance calcium-activated potassium channels (IK) in pancreas cancer cells (Jäger et al., 2004). Jäger and coworkers found elevated mRNA levels in 8 of 9 tested pancreatic tumor samples and the proliferation of IK-expressing pancreas cell lines was reduced in the presence of the IK-specific blockers clotrimazole or TRAM-34 (Jäger et al., 2004).

The general term non-Hodgkin lymphoma covers a group of more than 30 quite diverse subtypes that account for about 3% of all cancer incidences and death rates. Thus far, these cancers are only poorly examined with respect to ion channel profiles. Only recently, Smith and colleagues identified HERG channel expression in Burkitt's lymphoma cell lines (Smith et al., 2002). The cell lines Raji and BL2 showed a 6-fold increased mRNA level of HERG when compared to normal peripheral blood lymphocytes.

Malignant melanoma is the least common cancer type listed in Fig.1, but unfortunately incidence rates were characterized by a steady increase in the past four decades. Due to the relative ease of detection, this cancer is usually diagnosed at an early, localized stage with very good prognosis after surgical removal of the tumor. However, as soon as distant metastases occur, the five-year survival rate drops below 20% and new therapeutic strategies will be required to improve this situation. With respect to ion channels in this tumor entity, an early report by Nilius and Wohlrab described a prominent non-inactivating potassium current in the cell line IGR1 (Nilius & Wohlrab, 1992). A role of potassium channels in proliferation of IGR1 was suggested by the fact that the potassium channel blocker TEA inhibited the growth of the cell line. A later study on the same cell line identified the non-inactivating potassium channel as EAG1 (Meyer et al., 1999). In addition, IGR1 cells showed significant calcium-activated currents with characteristics of the IK channel, as well as volume-activated chloride currents (Meyer et al., 1999). Imipramine, a blocker of all three detected channel types, was found to inhibit proliferation in IGR1 cells (Gavrilova-Ruch et al., 2002). The calcium-activated IK channel was identified as a control element for melanoma cell migration (Schwab et al., 1999). In SKMEL28 cells, inhibition by charybdotoxin resulted in a strong reduction of migration rates. According to Tsavaler and colleagues, calcium-conducting TRPM8 channels can also be present in melanoma (Tsavaler et al., 2001) *In situ* hybridization of 4 melanoma tissue samples revealed 3

TRPMS-positive tumors. By contrast, the founding member of the TRPM subfamily, TRPM1 or melastatin, was significantly down-regulated in highly metastatic melanoma cells (Duncan et al., 1998). A detailed analysis of primary tumor material from 150 patients revealed the melastatin status as a potent prognostic marker for the development of metastases from primary melanoma. A loss of melastatin in primary tumors was significantly correlated with reduced 8-year disease-free survival rates (Duncan et al., 2001). Since TRPM1 is not characterized electrophysiologically, the functional role of this putative channel in tumor cells remains elusive, but control of intracellular $Ca^{2+}$ concentrations by TRPM1 has been suggested (Xu et al., 2001).

The overview given by Fig. 1 conveys three main impressions: (I) the number of ion channels that should be considered as putative new therapeutic targets is not too big; (II) most identified channels seem to be relevant in more than one cancer type, and (III), ion channels appear to have a higher prevalence in high-incidence cancers like colon, breast or prostate cancer, in comparison to cancers of the stomach, kidney or pancreas. The last point could certainly be biased by a very intensive electrophysiological analysis of some model cell lines like MCF-7 breast cancer cells, or the LNCaP prostate cancer cell line. In addition, Fig. 1 might underestimate the role of chloride channels in some tumors, as some described chloride currents could not be ascribed to specific proteins yet. Among the tumors with a suspected strong influence of chloride channels are the different types of gliomas. As most neurons lose their potential to proliferate, the majority of primary brain tumors is derived from supporting glia cells. The incidence rate for brain tumors is well below 3%, but surgical treatment is often compromised, as advanced gliomas disseminate rapidly throughout the brain. There is a growing body of evidence for an important role of volume-activated chloride channels, both, for the uncontrolled proliferation and for aggressive migration (Rouzaire-Dubois et al., 2000; Ransom et al., 2001). The molecular nature of these channels is still under debate, but recent studies argued for CIC-2 and CIC-3, two members of the voltage-gated chloride channel family, to be involved in the process of volume regulation (Olsen et al., 2003). Olsen and coworkers proposed that changes in cell size and shape can specifically facilitate the movement of glioma cells through narrow extracellular spaces in the brain. Glia cell-derived tumors are also unusual with respect to the voltage-gated potassium channel EAG1. While EAG1 channels are generally discussed as oncogenic factors being overexpressed in tumor tissue (Pardo et al., 1999), a recent analysis of normal brain, low-grade glioma and high-grade glioma revealed an inverse correlation between EAG1 expression and malignancy grade (Patt et al., 2004). The occurrence of EAG1 channels as shown in Fig. 1 may be an underestimate, as its expression in additional tumor types, including colon and prostate, has already been reported in scientific meetings and EAG1 functional expression has been confirmed for cervix carcinoma biopsies (Farias et al., 2004). For neuroblastoma, another cancer type with lower incidence, the functional expression of EAG1 channels has been demonstrated by electrophysiological analysis of the human cell line SH-SY5Y (Meyer & Heinemann, 1998). Regardless of these inevitable shortcomings of the ion channel profiles represented in Fig. 1., this list makes immediately obvious that the selection of ion channels is not arbitrary. Out of more than 80 known human potassium channel proteins, only 8 representatives appear as putative oncogenic factors. The same holds true for all other ion channel superfamilies, including the TRP channels with at least 20 members, but only 3 candidates for a distinct role in oncogenic transformation. This apparent specificity is a first prerequisite for the closer evaluation of ion channels as therapeutic targets.

## Ion Channels as Targets for Cancer Therapy?

Historically, the first line of evidence for a role of ion channels in cancer was based on the observation that ion channel blockers can inhibit cellular proliferation in vitro. Experiments of this kind were performed long before the molecular correlates of the analyzed currents could be identified. For potassium channels, an overview of proliferation experiments and the influence of channel blockade can be found in an extensive review by Wonderlin and Strobl (1996). The finding that inhibition of channel activity interferes with cancer cell growth was a first proof of principle for the usefulness of channels as therapeutic targets. Now that individual channel types can be assigned to specific cancer types, the race is open to search for more specific blockers and to evaluate them in clinical trials. It is no big surprise that TRPM8 and EAG1 were among the first targets that have been chosen by specialized biotech companies for intensive investigations. While many commercial efforts are now underway, some important questions are still waiting to be resolved. Do we really know whether the channels are active in the tumor? Any attempt to block a channel in tumor cells will only make sense if the channels have to conduct ions in order to promote tumor progression. For obvious technical reasons, the electrophysiological analysis of all described channel proteins has preferentially been done on model cell lines, while channel expression in tumors is routinely assayed by Western blotting, immunohistochemistry, RT-PCR, or in situ hybridization. It cannot be excluded that high mRNA levels of individual ion channels are not reflected in detectable

channel activity in the tumor cells. In principle, it is possible to perform electrophysiological analysis on slices of acutely isolated tumor samples, but this is a very demanding task and not practicable in clinical routine. Another possibility is the culture of primary cells from tumor tissue and analysis of the cells in the very first days after surgery. Using this approach, Farias and coworkers provided the first evidence for active EAG1 channels in cervical cancer cells (Farias et al., 2004). Functional analysis is further complicated by the fact that channels can be highly regulated, thus, media conditions used for isolated cells could have a substantial impact on channel activities. On the other hand, the microenvironment in the tumor, such as cell-cell contacts or oxygen deprivation in solid tumors could affect the channel activity in situ. To address the latter question we compared the activity of intermediate-conductance potassium channels (IK) in the melanoma cell line IGR1 under normoxic and hypoxic (3 % oxygen) conditions. Interestingly, current responses of this channel type were significantly enhanced by low oxygen conditions (Schönherr et al., 2005). Even if a specific channel is active in a tumor cell, this does not necessarily mean it is required for cell function. With the use of gene chips and genome-wide expression profiling it became evident that hundreds of genes can be differentially expressed in cancer cells, compared to normal cells. Thus, up-regulation of ion channels could be a simple coincidence, rather than a regulatory factor. As discussed before, the impact on cell growth in vitro has already been shown for many of the discussed channels, but data from clinical trials are now required to make assumptions about a potential clinical relevance of these findings. Most efforts to use ion channel blockers are still in the preclinical phase of development, but a large amount of data already exists for the long-term use of calcium channel blockers like verapamil as antihypertensive medication, and it should be instructive to search for correlations between such medications and the risk of cancer development. This has been done in a large prospective study on the long-term risk of prostate cancer in male users of calcium channel blockers, revealing a significant inverse association between prostate cancer and channel blocker use, implying that calcium channel blockers can lower the risk of developing prostate cancer (Debes et al., 2004). An effect of hypertension itself is unlikely, as a control group, using β-blockers instead of calcium channel blockers, showed no association to prostate cancer (Debes et al., 2004). However, this study also gave the surprising result that the effect of calcium channel blockers was strongly dependent on the family history for prostate cancer. For the total group of men enrolled in this study, the risk of prostate cancer was lower for blocker users (odds ratio 0.5) compared with nonusers. By contrast, among those men with a family history of prostate cancer, the likelihood to develop prostate cancer was significantly increased for calcium channel blocker users (odds ratio 2.64) compared with nonusers (Debes et al., 2004). Thus, the genetic background of an individual patient might be extremely important for help potential use of calcium channel blockers in cancer therapy. A molecular interpretation of these results is certainly not straightforward. It seems plausible that calcium channels in prostate cells are important for tumor formation, but verapamil has also been shown to block a potassium current in LNCaP prostate cancer cells (Rybalchenko et al., 2001). The advent of antagonists for other channel types in human trials is certainly slowed by a lack of highly selective compounds for some of the more recently discovered two-pore potassium channels, including KCNK9, for the TRP super family with TRPM8 and TRPV6, and for the subfamilies of chloride channels. A detailed discussion of the pharmacological profiles of putative cancer-related channels is out of scope for this overview but can be found in recent reviews (Wickenden, 2002; Nilius & Droogmans, 2003; Doering & Zamponi, 2003; Gudermann & Flockerzi, 2005). A very comprehensive collection of pharmacological data is also available in the IUPHAR compendium of voltage-gated ion channels that can be found at <http://www.iuphar-db.org/iuphar-ic/>. The further development of new and more specific blocking agents will certainly be accelerated by the availability of automated patch-clamp devices and by the increasing number of crystal structures of potassium channels that can guide the *in silico* refinement of new lead structures. However, there may be general limitations which are hard to overcome. A major hurdle for the therapeutic use of potassium channel blockers is the cardiac relevance of the HERG channel. Since the finding that mutations in the HERG gene as well as blockade of the channel can cause cardiac arrhythmia (long-QT syndrome), a long and growing list of drugs has been associated with HERG block and QT prolongation (Sanguinetti et al., 1995). This list does not only include antiarrhythmic drugs but also antihistamines, antipsychotics, antidepressants, antibiotics and other compound classes (De Ponti et al., 2002). This finding led to drug withdrawals and imposed new guidelines for the assessment of new drugs for QT liability. Does this mean that any use of potassium channel blockers in cancer therapy comes with an intrinsically high and unacceptable risk? Certainly not in general, but one potential therapeutic target, the HERG channel itself, seems out of reach. Moreover, no blockers of the closely related EAG1 channel are known yet with sufficient specificity for EAG1 over HERG. On the other hand, the fact that normal expression of EAG1, and many other voltage-gated channels, is mainly restricted to the brain makes them very interesting as potential targets. Specific

inhibitors that do not pass the blood brain barrier could be used for cancer therapy with lower risk for the patient. One possibility to overcome the close relationship between EAG1 and HERG may be the use of blocking monoclonal antibodies directed against EAG1. New channel blockers with increased clinical relevance of ion channels in cancer specificity are certainly desirable for a successful use in vivo. However, even old blockers with lower specificity are not fully excluded from in vivo trials. For example the classical potassium channel blocker 4-aminopyridine is currently assessed in a phase II clinical trial for its potential use in Guillain-Barre syndrome, a common cause of neuromuscular paralysis. Among the ion channels listed in Fig. 1, the intermediate-conductance calcium-activated channel (IK) might be one of the first in clinical trials on cancer. Potential drugs with high potency and specificity include ICA-17043 (Stocker et al., 2003) and TRAM-34 (Wulff et al., 2000), derivatives of the long known antifungal agent clotrimazole. ICA-17043 already reached a phase III clinical trial to investigate its effect in sickle cell disease. In erythrocytes, the IK channel (here termed Gardos channel) is involved in cell volume control and IK inhibition was found to prevent dehydration and sickling of red blood cells in vitro and in mice (Stocker et al., 2003). Together with voltage-gated Kv1.3 channels the intermediate-conductance channel is also an important modulator of T-cell-mediated immune responses and TRAM-34, as well as specific blockers for Kv1.3 are currently evaluated for their use as immunosuppressants (Chandy et al, 2004). These few examples show that ion channels are already used as therapeutic targets and the principal limitations of ion channels in cancer therapy may not be higher than for any other drug target. Apart from new small molecule blockers and blocking antibodies, the use of small interfering RNA (siRNA) may add an additional selective tool to interfere with ion channel activity in the future. In the first instance, this technique allows a safer target validation in the laboratory. In addition, the in vivo use of siRNA in cancer and other areas of disease is widely discussed (Izquierdo 2004; Tong et al., 2005). Human trials will have to define the real potentials and limitations of this promising new technique.

## New Prognostic Parameters?

Independent of the suitability of individual ion channels as therapeutic targets, the identification of an "ion channel profile" from tumor biopsies could certainly have a diagnostic value of its own. Analysis of a biopsy has given guidance for important decisions, but for many tumor classes the number of reliable markers is still low. The question is not only whether a malignant tumor is present or not, but rather which therapy is appropriate: Is the surgical removal required, will lymph node dissection be beneficial, or what is the risk of distant metastases? Classical parameters include histopathology, size of the tumor and family history. In the last years, new molecular markers could be identified for several cancer types and some of them revealed high potency and specificity. A prominent example is the recent finding that specific mutations in the epidermal growth factor receptor can predict whether a patient with non-small-cell lung cancer will respond to the tyrosine kinase inhibitor gefitinib (Lynch et al., 2004; Paez et al., 2004). Unfortunately, the list of candidate markers that could not be established as reliable factors is long and the search for indicators only at the beginning. As outlined before, expression of some ion channel proteins has already been analyzed in larger quantities of tumor samples and correlations to the tumor status could be delineated in some cases. One example is the loss of TRPM1 (melastatin) in malignant melanoma that predicts a poorer prognosis (Duncan et al., 2001). In a study on 150 patients with primary cutaneous melanoma the presence of TRPM1 in the tumor was evaluated by in situ hybridization and the presence of TRPM1 was associated with a significantly lower risk to develop metastases in a period of eight years after excision of the primary melanoma. The prognostic utility of TRPM1 was independent of other evaluated markers and a combination of TRPM1 status with classical staging parameters allowed a prediction on the disease-free 8-year survival. If diffuse TRPM1 expression was detected in stage II patients, the disease-free 8-year survival rate was 90 %, but this value dropped to 51 % if no TRPM1 was detectable in the primary tumor (Duncan et al., 2001). One of the main questions after excision of a primary melanoma is whether this should be followed by lymph node dissection or only by lymph node observation ("watch and wait"). Together with lymph node biopsy the TRPM1 state in the primary tumor may allow a safer prediction of lymph node metastases in the future. For most ion channel proteins discussed so far, a critical assessment in prospective studies is still required in order to judge their prognostic power. Detectable expression of some channels was closely correlated to the presence of a tumor, e.g. 16 of 16 tested prostate cancers were positive for TRPM8 (Tsavaler et al., 2001). In contrast, many other channels, expressed only in a smaller fraction of the analyzed tumor samples, will certainly be analyzed for the prognostic value of detectable expression. Good candidates that showed such correlations in preliminary investigations include GIRK1 for lung cancer (Takanami et al., 2004) and TRPV6 for prostate cancer (Fixemer et al., 2003). For TRPM8, a reduced expression in progressing stages of prostate cancer has been reported (Henshall et al., 2003), and it will be interesting to see

whether TRPM8 has a predictive potential in long-term studies. The examples discussed so far require the analysis of excised tumor tissue or biopsies by in situ hybridization, immunohistochemistry or similar techniques. In principle, it may also become feasible to use blood samples as a source to detect aberrant expression patterns of tumor-related genes. The progression of many tumor types is associated with the presence of circulating tumor cells in the peripheral blood of the patient. Detection and analysis of such cells is currently a very interesting topic, as new techniques like magnetic cell sorting based on nanoparticles have been developed, allowing the isolation of an extremely small cell population from the blood (Loberg et al., 2004; Braun & Naume, 2005). In addition to cell isolation, it is also possible to detect tumor-associated antigens in the blood by RT-PCR (Loberg et al., 2004), and it may be interesting to see whether mRNA detection of markers like TRPM8 from blood samples might have a predictive value.

In addition to the prognosis of disease-free survival and risk of metastases based on ion channel markers, any use of ion channels as therapeutic targets will directly require a test for this target channel in a given tumor. This follows the current trend to identify more specific markers for treatment susceptibility of individual cancers. Cancer therapy of the future is supposed to use complex information from each individual tumor, in order to define a patient-oriented regimen instead of a standard therapeutic strategy. The currently available data support the view that ion channels have the potential to strengthen both sides of cancer therapy, the highly individualized diagnosis, as well as a more patient-specific and targeted cancer treatment.

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