Voltage-Gated Ion Channels, New Targets in Anti-Cancer Research

Le Guennec Jean-Yves1,*, Ouadid-Ahidouch Halima2, Soriani Olivier3, Besson Pierre1, Ahidouch Ahmed2,4, Vandier Christophe1

1Inserm, E 211, Nutrition Croissance Cancer, Tours, France; Université François-Rabelais de Tours, Tours, France, 2EA 2086, Laboratoire de Physiologie Cellulaire et Moléculaire, Université Picardie Jules Verne, Amiens, France, 3CNRS UMR 6548, Nice, France; Université de Nice Sophia Antipolis, Nice, France, 4Laboratoire de Physiologie Animale, Faculté des Sciences, Université, Ibn-Zohr, BP 28/S, 80000 Agadir, Morocco

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Abstract: Cancer is one of the leading causes of mortality in the world. This is a complex disease involving many steps with proper signalling pathways. Early detection and treatment of cancers have increased survival and improved clinical outcome. However, novel strategies based on new interesting targets are needed to improve the conventional treatments. A few years ago, it appeared that some particular transmembrane proteins, ion channels, may be involved in the development of the disease. Since then, their role in cancer cell properties such as proliferation, migration and invasion begins to be unravelled. These proteins have been widely studied in non cancerous cells as well as in pathologies involving excitable cells and thus, their pharmacology are quite well known. In this review, we summarize the present knowledge about the role of different ion channels in some aspects of the development of tumours, mainly proliferation, migration and invasion. A particular emphasis is done on promising new patents.

Keywords: Cancer, proliferation, migration, invasion, calcium channels, CaV, potassium channels, Kv1.1, hEAG, hIK Ca, sodium channels, NaV, sigma receptors, SK3.

INTRODUCTION

Cancers are among the leading causes of death in the world and as such represent a major problem of public health. This is the reason why governments, pharmaceutical companies, industrial and academic researchers are all so intensely involved in a better understanding of the aetiologies of the diseases. Such efforts aim at the improvement in the early detection of tumours in order to propose more efficient treatments. These could be effective in replacement of or in association with other therapies used presently. One of the new promising fields of investigation is the pharmacology of ion channels which are known to be involved in different aspects of the carcinogenic process. Thus, it has been proposed to identify cancer tissues (mainly in lung) using electrophysiological methods [1].

All living cells have a difference of electrical potential between the inner and the outer side which is called the membrane potential. This membrane potential is generally due to the activity of ion channels. Ion channels are transmembrane proteins which allow ions to flow in and out of the cells, more or less selectively (see Fig. 1). As such, these proteins are very much involved in the communication between cells. Also, they play an important role in the regulation of cellular ionic homeostasis. For example, the vital function of sodium reabsorption in the kidney is performed thanks to ion channels called Sodium Epithelial Channels [2]. But this is not their sole role. Indeed, in other tissues the inward or outward flow of ions through the channels is accompanied by the generation of an electrical current. This current can modify the membrane potential in different directions and with different speeds. When the current is fast and important it can generate waves of depolarisation called action potentials. But when the changes in membrane potential are slower, the resulting changes of electrochemical gradients for ions can regulate the fluxes of these ions through pathways such as channels or exchangers. Up to now, hundreds of channels have been described. They can be classified according to the species of ions which pass through the channels, mainly sodium, calcium, potassium, protons and chloride. Their classification can also be based on the stimulus which triggers the opening of the channels. Among various stimuli, changes in membrane potential can regulate the activity of ion channels called Voltage-Gated Channels (VGC). These channels generally open when the membrane potential depolarises (in a few exception, channels open when the membrane potential hyperpolarises). This opening is permitted by the existence of a transmembrane segment of the protein which is rich in positively charged amino acids. The change in membrane potential induces a conformational movement of the protein which can be compared to the movement of a camera shutter. These channels have been particularly studied in excitable cells such as neurons or cardiac cells where they participate in the action potential.

To investigate the expression of ion channels, techniques such as RT-PCR or immunoblot allow determining the presence of mRNA or show their translation into proteins. However, one state-of-the-art technique exists, namely the patch-clamp technique [3], which allows to quantify the activity of these proteins. Displace-
ment of ions from one compartment to another leads to the generation of a current due to the electric charges borne by the ions. The patch-clamp technique uses a glass pipette as an electrode to measure the electric current flowing through the ion channels (see Fig. 2). Since the ionic flux depends mainly on passive diffusion, changes in current amplitude directly reflect the changes in channel conformation and thus activity. The two major configurations to measure currents flowing through all the channels of an isolated cell, and thus called whole-cell recording, are the "ruptured patch" (the membrane patch at the tip of the pipette is broken and the intracellular content is replaced by the medium present in the pipette) and the "perforated patch" (antibiotics such as amphotericin B present in the pipette medium form pores in the membrane trapped at the tip of the pipette). This latter configuration allows accessing electrically to the interior of the cell while reducing the dilution of intracellular soluble molecules.

**Fig. (2). Schematic drawing of the patch-clamp technique in Whole Cell Recording.** Left, the membrane patch under the pipette has been broken giving an electrical and physical access to the interior of the cell. The pipette medium diffuses into the cell. Right, Perforated patch condition. The pipette medium contains a ionophore (for example amphotericin B, which is a Na+ and K+ ionophore). This gives an electrical access to the interior of the cell since only ions passing through the ionophores can enter or flow out of the cell.

Many channels are known to be involved in cellular properties which participate in the oncogenic development. For example, potassium channels activities like hEAG, Kv1.1 or hKv4 (also called SK4) permit a more efficient cell cycle and thus a better proliferation. Their activities can induce the activation of over-expressed calcium channels or facilitate calcium entry through increase of the driving force. Consequently, they can change homeostasis of intracellular calcium.

Beside proliferation, cancer cells are able to digest the basal lamina and migrate into the blood and lymphatic circulation. From there, they will arrive at an appropriate organ in which they can develop a secondary tumour. This second step implies again that cells digest the extracellular matrix and migrate to nest and proliferate. It is known that migration can be promoted by an increase in intracellular calcium. This event can be controlled by potassium channels like SK3 [4]. This channel is not purely voltage-dependent but its activity can control the membrane potential which in turn will affect the activity of other transmembrane proteins leading to changes in cellular metabolism.

However, in order to migrate, cells first have to invade, i.e. to digest the extracellular matrix. This can be performed by proteases like matrix metalloproteases (MMP) [5] or cysteine cathepsins [6]. Over the last ten years, there has been a growing body of evidence that voltage-gated sodium channels (Nav) potentiate the in vitro invasivity of cancer cells [7]. These channels have been found in cell lines and biopsies of many cancers. They are thought to regulate proteases via molecular and cellular mechanisms which have to be determined [7]. This over-expression of Nav is rather surprising since these channels are usually found in excitable cells where they are responsible of the rapid phase of depolarisation of the action potential [8]. Whatever the way through which they promote the oncogenic properties of cells, it is clear that they are interesting targets to slow and even to prevent the occurrence of metastases.

In this review, we will describe three families of channels involved in epithelial cancers (with a particular emphasis on breast cancer), namely potassium, calcium and sodium channels. These channels can be regulated by sigma receptors, a particular class of nuclear receptors known to be affected during the development of cancer tumours. Since the regulation of sigma receptors is quite new in the field of ion channels and cancer, a special paragraph is devoted.

**POTASSIUM CHANNELS**

**Potassium Channels and Proliferation**

Numerous reports show that progression through the cell cycle is dependent on ion translocations across the plasma membrane. Thus, pharmacological blockades of K+ channels lead to cell proliferation inhibition [9-14]. Several studies have demonstrated that K+ channel activity is also a determining factor for cell progression through the G1 phase of the cell cycle [9,10,15]. Another relationship between K+ channels and cell cycle is indicated by the finding that the activities of some K+ channels change cyclically as cells progress through the division cycle. Czanecki et al. [16] have also reported an up-regulation of the K+ current in quiescent (G0 phase) compared with proliferating cells of the GH3 pituitary cell line. Furthermore, Kv1.4 and Kv4 α-subunits are responsible for the K+ current in GH3 cells. In the MCF-7 breast cancer cell line, two groups have reported that both proliferation control and cell cycle progression depend on K+ channel-activity according to the "membrane potential" model [17,18]. Thus, the inhibition of proliferation by K+ channel blockers is due to membrane depolarization. On the basis of electrophysiological studies, it has been suggested that at least 6 types of K+ currents, with differing degrees of dependence on voltage, intracellular Ca2+, and ATP, are expressed in human breast cancer cells MCF-7 [18-23]. In this paragraph, we will review the mechanisms by which K+ channels control the progression through the cell cycle of breast cancer cells, focusing on events in G1, and then discuss the deregulation of K+ channels in cancer.

**Electrophysiological and Pharmacological Properties of K+ Channels in MCF-7 Cells**

**KATP Channels**

Based on the pharmacological study on cell culture, the group of W. Wonderlin has reported that KATP channels (which are inhibited by glibenclamide and quinidine) are responsible for progression through the cell cycle [17]. In 1999, Klimatchiva and Wonderlin, by using whole-cell recordings, recorded a large macroscopic current in MCF-7 cells and fitted this current to the sum of three currents, namely: linear hyperpolarized, linear depolarized and outwardly-rectifying currents [20]. Different pharmacological interventions led to the conclusions that the linear hyperpolarized current was an ATP-sensitive K+ current which was required for the progression of MCF-7 cells through the G1 phase. Indeed, quinidine induced cell cycle arrest in early G1.

**Kv 1.1**

Epithelial cells from various organs express many Kv channels [13,24]. Among voltage-activated K+ channels involved in cell proliferation, Kv 1.3 is probably the best described [25,26]. However, in breast cancer cell line MCF-7, Kv 1.3 was not expressed. Only Kv 1.1 mRNA was detected [21]. A TEA-sensitive outward current similar to that reported by Klimatchiva and Wonderlin in MCF-7 cells [1999] was found [20]. This macroscopic outward current contained a non-inactivating K+ current which was α-dendrotoxin-sensitive (α-DTX) and exhibited a
threshold activation voltage of ~20 mV and did not inactivate. Inhibition of Kv1.1 activity by α-DTX reduced cell proliferation [21].

**Human Ether a Go Go**

Human ether à go-go (hEAG) K⁺ channels are reported to have oncogenic properties [14,27,28]. Their distribution is restricted to the brain in normal tissue, while it becomes ubiquitous in tumour cells [14,28,29]. Indeed, mRNA is expressed in cervical cancer cells, breast tumour, neuroblastoma, melanoma and colonic carcinoma cells [18,27,30,31]. Moreover hEAG has been suggested as being important for tumour cell proliferation [13,27,30,32]. Inhibition of hEAG expression (with antisense oligonucleotides, siRNA, imipramine, astemizole and quinidine) reduces cell proliferation in cancer cell lines [13,33,34]. Using RT-PCR, mRNA for h-EAG K⁺ channel was revealed in MCF-7 cells [18,27]. The inhibition of hEAG reduced MCF-7 cell proliferation and led to the accumulation of cells in the G1 phase [18,19]. The potential problem with these channels is that they belong to the same family as hERG which is a channel involved in cardiac repolarisation. As such, they are blocked by similar compounds. The blockade of hERG can induce cardiac arrhythmias and preclinical studies of newly developed molecules are performed to prevent this problem [35]. It is why the best strategy could be to use of antibodies directed towards specific isoforms of the channel in order to detect and cure the disease [36].

**BIG and Intermediate Ca²⁺ Activated K⁺ Channels**

Calcium-activated K⁺ channels have historically been subdivided into three distinct classes based on conductance and pharmacology. These include the large-conductance (BKCa) channels, which are sensitive to charybdotoxin (ChTX) and iberiotoxin (IbTX) blocking [37-39], the intermediate-conductance (hIKCa) channels, which are inhibited by ChTX, clotrimazole and TRAM-34 [37,40-42], and the small-conductance (SKCa), apamin-sensitive (and -insensitive) K⁺ channels [37,41]. Wegman et al., 1991 have previously reported that the MCF-7 cell line expresses a 23 pS Ca²⁺ and voltage-activated K⁺ conductance which is not blocked by TEA at 10 mM [19]. Based on the pharmacological profile, the BKCa channels were characterized in MCF-7 cells. The current was blocked by r-iberiotoxin (r-IbTX), Charybdotoxin (ChTX), and when cesium was applied internally. The BKCa currents were active at typical resting potentials (from -50 to -30 mV) with [Ca²⁺] near 500 nM [23,42]. In addition to the BKCa current, we have also characterized the intermediate Ca²⁺-activated current in MCF-7 cells. Indeed, these channels also mediate some of the outward native currents. These channels expressed in MCF-7 cells are not the typical Ca²⁺-dependent IK channels (hIKCa). The channel reported is sensitive to clotrimazole at 1 μM, TRAM-34 and to Ba²⁺, but not to ChTX [22]. The absence of effect of ChTX on hIKCa is an unexpected finding, since ChTX is known to be a classical channel blocker. Moreover, perfusion of the hIKCa activators chlorozoxazone and zoxazolamine activated the outward current recorded in MCF-7 cells and induced a hyperpolarization as expected for K⁺ channel openers. However, a prolonged exposure (3 days) of MCF-7 cells to chlorozoxazone (CZ) or zoxazolamine (ZOX) inhibited cell proliferation. Koege et al., [43] have reported a down-regulation of both hIKCa mRNA levels and channel activity after 3 days of treatment of HaCaT keratinocytes with hIKCa openers (I-EBIO, CZ and ZOX). Moreover, the lack of sensitivity of the outward current to the bee venom peptide toxin, apamin, or the plant alkaloid, d-tubocurarine, indicated an absence of small conductance channels (SK2, SK3) in MCF-7 cells [23]. However, Potier et al., [4] have recently reported that the expression and the activation of SK3 channel are associated with breast cancer cell migration in a more invasive cell line (see paragraph "Potassium channels and migration").

**K⁺ Channels Involved in Cell Proliferation and Cell Cycle Progression**

In the breast cancer cell line MCF-7, it has been reported that proliferation control and cell cycle progression depend on K⁺ channel activity according to the "membrane potential" model [9,18]. Thus, the inhibition of proliferation by K⁺ channel blockers is due to the depolarization of the membrane. Wonderlin et al. [17] suggested that the hyperpolarization during the transition through G0/G1 and into the S phase probably results from an increase in the relative permeability of the plasma membrane to K⁺. In MCF-7 cells, a linear hyperpolarized ATP-inhibited K⁺ current [20], Kv1.1 K⁺ current [21], BKCa [23], hIKCa [22], and a hEAG K⁺ current [18] have been characterized. With the exception of the BKCa channel, all the others are involved in the control of proliferation (see Table 1). The ATP-sensitive, hEAG, and hIKCa K⁺ channels are required for the cell to proceed through the G1 phase [17,18,44].

Table 1. Different Potassium Channels Involved in Breast Cancer and their Implication in Cell Proliferation (+++: High Involvement; ++: Good Involvement; -: No Involvement) as Assayed by their Blockade by the Drug Indicated

<table>
<thead>
<tr>
<th>Current/channel</th>
<th>Drugs</th>
<th>Proliferation</th>
<th>References</th>
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<tr>
<td>23-pS Ca²⁺-activated</td>
<td>Quinidine</td>
<td>+++</td>
<td>[19]</td>
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<tr>
<td>KATP</td>
<td>Quinidine</td>
<td>++</td>
<td>[17, 20, 44]</td>
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<tr>
<td>Kv1.1</td>
<td>α-DTX</td>
<td>++</td>
<td>[21]</td>
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<tr>
<td>hEAG</td>
<td>Astemisole</td>
<td>+++</td>
<td>[18]</td>
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<td>BKCa</td>
<td>r-IbTX</td>
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<td>ChTX</td>
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<td>hIKCa</td>
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**Role of Kv 1.1 and BKCa Channels in Cell Proliferation**

The expression of BKCa channel is not constant in MCF-7 cells throughout the cell cycle. The current-densities of BKCa are not detected in cells arrested in the early G1, increase in cells arrested in the late G1 and in the S phase. These changes are consistent with RT-PCR studies showing an increase in BKCa mRNA levels in the S phase. Treating MCF-7 cells with BKCa-inhibitors (r-IbTX or ChTX) induced a weak depolarization and had no effect on cell proliferation. Thus, BKCa K⁺ channels seem to be only slightly involved in the regulation of the membrane potential, at late G1 and S phases, when the BKCa current-density was shown to increase. This increase in the BKCa current-density might be due to a serum effect. Indeed, serum and growth factors have been reported to up-regulate K⁺ channels in human intestinal smooth muscle cells [45], and myeloblastic cells [46]. As MCF-7 cells express numerous K⁺ channels, serum could activate some of them and thereby induce sufficient hyperpolarization to stimulate cell proliferation. In MCF-7 cells, serum starvation induces an inhibition of cell proliferation, cell arrest in the G1 phase, and membrane depolarization [18]. Thus, the failure of r-IbTX to effect membrane potential or cell proliferation, suggests that the membrane potential is mainly...
determined by another K⁺ channel type. Moreover, the absence of r- IbTX effect on cell proliferation may suggest that, under resting conditions, the intracellular free Ca²⁺ level is too low to activate r- IbTX-sensitive, Ca²⁺-activated K⁺ channels. This conclusion is in agreement with the results of de-Allie et al. [47] showing that Ca²⁺-activated K⁺ channels are inactive in the presence of a basal cytosolic Ca²⁺ of 42 nM. We found that intracellular [Ca²⁺]ᵢ levels were lower in those MCF-7 arrested in the early G1 phase (48 nm). Similar results were reported by Roger et al., [42], who found that inhibition of BKCa channels by IbTX was without effect on MCF-7 cell proliferation. However, when [Ca²⁺]ᵢ is increased by the extracellular addition of ATP, IbTX reduces cell proliferation [42]. This finding can be related with observations that the gene coding for the BKCa (called KCNMA1) is up-regulated in some metastatic prostate cancer biopsies and as such is a potential new target [48].

**How Might Both hEAG and hIKCa Channels Contribute to MCF-7 Progression Through the Cell Cycle? Membrane Potential Model**

In 2001, it has been shown that the outwardly-rectifying, TEA-sensitive current controls membrane potential and induces the release of cells from G0 [18]. In MCF-7 cells, evidences were provided that membrane hyperpolarization is necessary for cell cycle progression [17,18]. The average resting membrane potential (RMP) values measured in MCF-7 cells by Ouadid-Ahidouch et al. [18] were similar to the ~40 mV measured by Wegman et al [19] and Marino et al. [49], but more hyperpolarized than the ~23 mV measured by Wonderlin et al. [17]. Comparison of RMP distributions of cells arrested in G0/G1 and cells progressing through the G1 or S phases clearly indicated that RMP hyperpolarizes during the G0/G1 phase transition. The RMP in MCF-7 cells was determined by the K⁺ current density. Iₖ⁺ density was much higher in the G1 and S phases, as compared with cells arrested in G0/G1, suggesting the involvement of K⁺ channels in controlling the cell cycle [18]. Wang et al [50] have shown that an increase in the relative permeability to K⁺ (by treatment with the K⁺ ionophore valinomycin) can counterbalance the arrest of MCF-7 cells in G1 phase by the nonspecific K⁺ channel blocker quinidine, thus indicating the role of K⁺ permeability in MCF-7 cell cycle control. Wonderlin et al [17] have suggested that the hyperpolarization during passage through G0/G1 and into the S phase probably results from an increase in the relative permeability of the plasma membrane to K⁺. In MCF-7 cells, firstly, a Ca²⁺-voltage-activated K⁺ current [19] rather than a linear hyperpolarized ATP-inhibited K⁺ current [20] has been suggested to be involved in proliferation control. A 23 pS K⁺ current was found to be more active during logarithmic growth in MCF-7 cells and the ATP-sensitive one was required to progress through G0/G1 and into S phase [9,44]. Our group revealed that the expression of mRNA of the hEAG K⁺ channel in MCF-7 is strongly regulated during the cell-cycle progression [18].

Treatment MCF-7 cells with a K⁺ channel inhibitor (TEA) or EAG inhibitors (astemizole or siRNA) reduced cell proliferation in a dose dependent manner, and led to the accumulation of cells in the G1 phase along with a reduction of the number of cells in the S phase [18,51]. hEAG channels also appear to play a critical role in the life of cells [52]. Moreover, in human neuroblasts, hEAG channels are only expressed in undifferentiated cells, thereby indicating a very tight coupling of hEAG expression to the cell cycle.

We went on to show that the activation of hEAG K⁺ channels, which induced a hyperpolarization of the membrane potential and progression through the early G1 phase, was transient [18]. Strikingly, the hyperpolarization of membrane potential continued at the ends of both the G1 and S phases, while the hEAG K⁺ current density decreased. hIKCa current-density dramatically augmented in cells at the end of G1 and in those entering the S phase, in parallel with a strong hyperpolarization. Pharmacologically blocking hEAG channels has been shown to depolarize cells both when they were accumulated in early G1 and when they were progressing through G1 phase. In contrast, blocking hIKCa induced a depolarization only in cells arrested at the end of the G1 and S phases.

**Correlation Between Membrane Potential, hEAG, hIKCa Channel-Activity, and Intracellular [Ca²⁺]ᵢ**

Changes in the cytosolic calcium concentration [Ca²⁺]ᵢ may also provide important regulatory signals during the cell cycle. Ca²⁺ has been observed to be required for progression through G1 and for the G1/S transition in several cell types [53-57]. A link between [Ca²⁺]ᵢ and membrane potential was first reported in melanoma cells, where membrane hyperpolarization increases [Ca²⁺]ᵢ simply by controlling the electrochemical gradient for Ca²⁺ entry into the cell [58]. This increase in [Ca²⁺]ᵢ may, in turn, induce the activation of Ca²⁺-activated K⁺ channels (KᵥCa). In MCF-7, the inhibition of hEAG reduces cytosolic [Ca²⁺]ᵢ in those cells arrested in the early G1 phase, while the inhibition of hIKCa induces a greater decrease in [Ca²⁺]ᵢ in cells arrested at the end of G1 and throughout the S phases. We showed a direct correlation between membrane depolarization and the decrease in the basal [Ca²⁺]ᵢ. Thus, inhibition of K⁺ channels and the decrease in [Ca²⁺]ᵢ represent a possible means of specifically inhibiting MCF-7 cell proliferation.

**Relation Between hEAG and hIKCa Channels, Calmodulin and Membrane Potential**

Several studies have reported that hIKCa activity may be dynamically regulated by phosphorylation [40,59-61]. Interestingly, both hEAG and hIKCa channels are regulated by intracellular Ca²⁺ and calmodulin (CaM) [40,62]. [Ca²⁺]ᵢ reduces hEAG and increases hIKCa channel activity. This apparent paradox is explained by the various regulatory mechanisms of the system: hEAG channels are closed by the binding of only one CaM molecule [62], whereas it has been reported that Ca²⁺-loaded CaM molecules are required to activate hIKCa channels [63,64]. The reverse regulation can take place in the same cell, for example, in human melanoma, where both hEAG and hIKCa channels have been identified [30]. In MCF-7, perfusion of 10 μM W-7, a CaM inhibitor, increased hEAG current-density and hyperpolarized the membrane potential, whilst it reduced hIKCa current-density, inducing membrane depolarization (unpublished observation). These results suggest that the phosphorylation-dependent modulation of hEAG and hIKCa plays a critical role in modulating the progression of cells through G1 and into the S phase. Moreover, in breast cancer cells, it has been reported that Ca²⁺ is involved in controlling cell growth through its interaction with calmodulin [65]. Furthermore, MCF-7 cells require calmodulin to traverse the G1/S boundary [66-68]. Deficiency of MCF-7 cells with CaM antagonists (calmidazolium and W-12) inhibits proliferation and causes an increase in the percentage of cells in the G1 phase, accompanied by a decrease in the percentage in the S phase [68]. Recently, it has been reported that CaM-Kinase Kinase (CaMK-KK) and CaM-Kinase I (CaM-KI) participate in the control of the G0/G1 restriction checkpoint of the MCF-7 cell cycle. Inhibition of both CaM-KI and CaM-KK by specific interfering RNA causes an arrest in the G1 phase of the cell cycle. This arrest seems to be due to an inhibition in cyclin D1 synthesis and a reduction in pRb phosphorylation [69].

**Both hEAG and hIKCa Channels Contribute to MCF-7 Cell Progression Through the Cell Cycle**

The ability of the hEAG blockers to inhibit proliferation and to arrest the cell cycle indicates that this type of K⁺ channel may play a critical regulatory role in the growth of human breast cancer cells. However, as hEAG expression is transient and the membrane potential continues to hyperpolarize through the G1 and S phases, we propose a detailed model in Fig (3) that links the activity of these two classes of K⁺ channels. In early G1, the membrane potential is depolarized (~ -20 mV) with little or no hIKCa activation, due perhaps to a low resting [Ca²⁺]ᵢ. hEAG is voltage-gated, activated by depolarization, and its steady-state activity is not
insignificant at -20 mV. A neo-expression of hEAG channels (increase in mRNA levels) induces an increase in current-density, hence hyperpolarizing the membrane potential and increasing Ca\textsuperscript{2+} entry. Gating of the hIK\textsubscript{Ca} channel is voltage-independent but hyper-sensitive to increases in internal Ca\textsuperscript{2+}. Thus, the initial Ca\textsuperscript{2+} entry during G1 is regeneratively amplified by the activation of hIK\textsubscript{Ca} channels, resulting in the strong hyperpolarization of the membrane potential during progression through G1 and into the S phase. The over-expression of hEAG may be regulated by growth factors or estrogens. Indeed, several studies have reported that growth factors such as insulin, IGF-1 and EGF stimulate cell proliferation and up-regulate the expression of several K\textsuperscript{+} channels [70-72]. Recently, we have reported that IGF-1 activates MCF-7 cell proliferation by up-regulating the expression of hEAG. Specific inhibition of hEAG by siRNA inhibited the IGF-1 mitogenic effect [51].

K\textsuperscript{+} Channels and Breast Tumourogenesis

All studies carried out on breast tissues show an over-expression of K\textsuperscript{+} channels. EAG channels are over-expressed in many tumours including breast cancer [29]. KCNK9 encodes a TASK potassium channel that is amplified from 3-fold to 10-fold in 10% of breast tumours and is over-expressed by 5-fold to over 100-fold in 44% of breast tumours [73]. Over-expression of KCNK9 in cell lines promotes tumour formation and confers resistance to both hypoxia and serum deprivation. They suggest that TASK may contribute to tumourigenesis by promoting cancer cell survival in the poorly oxygenated areas of solid tumours [73]. Thus, KCNK9 can be a marker of such cancer [74]. Stringer et al., [75] 2001 have measured the G-protein dependent inwardly-rectifying potassium channel GIRK1 mRNA expression in benign breast tumour tissue, primary invasive breast carcinomas, and metastatic breast carcinomas from axillary lymph nodes using quantitative TaqMan reverse transcription-PCR and correlated their results with clinical parameters. They found that GIRK1 over-expression correlated with lymph node metastasis and over-expression was greatest in tumours with more than one positive lymph node. Their results indicate that GIRK1 may be useful as a biomarker for lymph node metastasis and possibly as a pharmaceutical target [76]. Using immunohisto-chemistry, Abdul et al., [77] reported an over-expression of Kv1.3 in breast carcinomas. Recently, the same authors observed a reduction of the expression of Kv1.3 in epithelial prostatic cancer cells studied in immunostaining [78].

We have performed immunohistochemical analysis on 33 primary human breast cancer specimens, 31 normal human breast specimens and 30 hyperplasic human breast specimens. In breast cancer tissue, we showed an over-expression of GIRK1 K\textsuperscript{+} channels and a strong reduction in Kv 1.1 and Kv 1.3 expression in comparison to normal tissue. This latter result is in complete accordance with those in prostatic cancer cells.

Potassium Channels and Migration

Cell migration is an important physiological process throughout life and persists in pathophysiological processes. Migrating tumour cells are in part responsible for death in cancer since tumour cell migration is one of the crucial steps in the metastatic cascade. A migrating cell is polarized within the plane of movement [79,80]. The front is formed by pseudopods which drive the protrusion of the cell. The retraction of the rear end of the migrating cell is thought to be related to the contraction of the acto-myosin network
This functional polarization of cytoskeletal structures is also maintained by a gradient of calcium concentration which is higher at the rear end than in the pseudopods [81]. The function of potassium channels is closely related to the actin cytoskeleton which is, in turn, regulated by the state of actin filaments.

Among all potassium channels only a few have been linked to cell migration [82]. Kv 1.1 and Kv 1.5 have been involved in the migration of both non cancer and cancer cells while IKCa, BKCa, and Kv 1.3 have been shown to regulate the migration of cancer cells only [82]. In human melanoma, Kv 1.3 channels physically interact with β1-integrins and thereby control cell adhesion [83,84]. A prominent role in migration is played by calcium-activated potassium channels (KCa). As already described (in paragraph Potassium channels and proliferation, big and intermediate Ca2+-activated K+ channels), KCa channels comprise many channels which differ in their primary amino acid sequences and exhibit different single channel conductances and pharmacological profiles. Among KCa channels, IKCa channel (also named SK4 channel) appears to be involved in the migration process of cancer and non cancer cells [82]. This channel facilitates the retraction of the rear part of migrating cells by inducing local cell shrinkage at this cell pole [82]. Due to the intracellular gradient of calcium, SK4 channel seems to be active mainly at the rear part of migrating cells. Nevertheless, SK4 and another KCa channel (SK3 channel) have been shown to be concentrated in the front part of the migrating cells [85,86]. The apparent discrepancy between calcium gradient and KCa localisation remain to be elucidated. The role of BKCa channel in cancer cell migration is less clear or is tissue specific. If BKCa channels activation reduce migration of some cancer cells, these channels are not involved in the migration of breast cancer cells [23,42,87].

Recently, another KCa channel, SK3 channel, has been involved in breast cancer cell migration [4]. Three isoforms of SKCa α subunits, named SK1, SK2 and SK3, can associate to form homo- or hetero-tetramers [88,89]. SK3 protein is principally expressed in central neurons where SK3 channel contributes to the long lasting after-hyperpolarisation that follows an action potential [90]. If a decrease in SK3 expression seems to be involved in schizophrenia, SK3 expression increases with age in the hippocampal formation, thereby contributing in hippocampal long-term potentiation [91]. SK3 protein expression was shown in vascular and visceral smooth muscle-rich tissues [92]. For example, SK3 protein was observed in the endothelium of vascular smooth muscles, in smooth muscle cells and in the endothelium of urinary bladder. In these muscles, the activation of SK3 channels induces a hyperpolarisation which regulates muscle tone [92-96]. SK3 expression was also detected in cavernosal smooth muscles, in hepatocytes, in pancreatic cells, and in fibroblast-like cells of the gastro-intestinal tract [97-101]. The role of SK3 channels in these structures remains to be elucidated. We recently reported that SK3 protein is also expressed in a highly metastasizing mammary cancer cell line, MDA-MB-435s, where it is involved in cell migration but not in cell proliferation [4]. SK3 protein expression was only observed in these cancer cells in contrast to SK2 protein which was expressed both in cancer and non-cancer cell lines. While siRNA directed against SK3 almost totally abolished MDA-MB-435s cell migration, transient expression of SK3 increased the migration of the SK3-deficient cells. Thus, SK3 channel appears as a new mediator of breast cancer cell migration [4]. Hence, we proposed that SK3-mediated cancer cell migration could be used as a method for inhibiting the migratory, invasive and metastatic properties of cells expressing SK3 channel for the treatment of cancers such as melanomas, and breast cancer [102]. The patent further relates to drug screening methods designed to identify compounds that inhibit SK3 activity and the use of such compounds in the treatment of cancers.

Numerous reports have suggested the possibility that SK3 protein forms a heteromeric channel with the other SKCa channel subunits SK1 and SK2 [88-89]. Since SK1 gene is not expressed in MDA-MB-435s cells, assembly of either SK3 proteins or SK3 and SK2 proteins, resulting in respectively SK3 homomeric and SK2/SK3 heteromeric channels, may form the functional SKCa channels involved in cell migration. Further experiments are necessary to determine the precise structure of the functional SK3 channel effective in cell migration. Since SK3 channel hyper-polarizes the cell membrane of MDA-MB-435s cells and that a 24-hour apamin treatment decreases the intracellular calcium concentration of MDA-MB-435s cells, we propose that SK3 channel promotes cancer cell migration by hyperpolarising the cell membrane, leading to calcium entry through existing voltage-independent calcium channels. Thus, SK3 channel appears to be a low-energy-cost modulator of cell migration. Therefore, a positive feedback loop exists in which Ca2+ entry through Ca2+ channels increases the activity of SK3 channel, leading to a more negative membrane potential, which results in a stronger electrochemical driving force for Ca2+ and an enhancement of Ca2+ entry through voltage-independent Ca2+ channels.

What Must be Remembered about Potassium Channels and Cancer?

We conclude that cyclical hEAG and hIKCa channel activity is necessary to drive the progression in the cell cycle of MCF-7 cells. GIRK1 might also been involved. With SK3, these channels are probably the most interesting targets to inhibit in order to fight cancer.

However, many fundamental queries remain about the regulation of breast cells' potassium channels: (1) Do TRP channels (ion channels not controlled by voltage) play a role in Ca2+ influx? (2) Does any endogenous ligand regulate K+ channels? (3) Which are the physiological roles of CaM, CaM-Kinases and Ca2+ in the control of native K+ channels? (4) Is there any regulation of cyclins, cyclin-dependent kinase inhibitors or cyclin-dependent kinase stimulators by K+ channels?

As hEAG and hIKCa K+ channels are both involved in cell cycle progression and have an oncogenic potential, this has granted us a promising and exciting message about the potential application of hEAG and/or hIKCa as a target for cancer therapy in general and breast cancer in particular.

SK3 seems to be preferentially involved in cell migration. Why can different channels, which in turn regulate calcium homeostasis, have such different cellular functions?

CALCIUM CHANNELS

Voltage-gated calcium channels (VGCaC) have an important role in the regulation of calcium homeostasis and thus in contraction, secretion and gene regulation. They possess a main α subunit of high molecular weight (approximately 250 kDa) associated or not with auxiliary subunits. Up to now, ten genes encoding the α-subunit have been identified and the nomenclature used is rather confusing. The oldest one refers to the current produced by the inward flow of calcium through the channels (ICaL, ICaT, ICaN, ICaP, ICaQ, ICaR) and the pharmacology. With the cloning of the channels, a more precise nomenclature appeared for the α subunits being named by αi followed by a letter which initially was the first letter of the name of the tissue where the channel had been found (α1S in skeletal muscle, α1C in cardiac muscle). The habit of linking the tissue and the letter was not kept on and letters were then added following the alphabetical order (α1A, α1B, ...) with no reference to the tissue. At the beginning of the century, a new nomenclature, similar to that used for potassium channels, has been proposed. Three families of channels have been distinguished. The rationale for this nomenclature is first based on the pharmacological and electrophysiological properties of the channels. Some channels need a larger depolarisation to be open than others. The former are designated as High-Voltage-Activated (HVA), the latter Low-
Voltage-Gated Ion Channels, New Targets in Anti-Cancer Research

Voltage-Activated (LVA). The HVA are divided in two families of channels based on their sensitivity to dihydropyridine (DHP): those which are sensitive to micromolar concentrations of DHP are called DHP sensitive and those which are not sensitive to DHP at these concentrations are called DHP insensitive. The families of the α-subunits are: Cav-1 for the HVA-DHP sensitive, Cav-2 for the HVA-DHP insensitive and Cav-3 for LVA. In this latter family, three channels are known: Cav-3.1 (α1G in the former nomenclature), Cav-3.2 (α1H) and Cav-3.3 (α1J). All these proteins are responsible of currents known as ICaL. No auxiliary subunit is known to be associated with these α-subunits. The reference blocker of these channels is presently mibefradil but this inhibitor is not selective for Cav-3 channels. The Cav-3 proteins and ICaL associated currents have been widely studied in non-cardiac cells where they participate in the regulation of calcium homeostasis and in the cellular excitability. However, it has been shown in other tissues such as smooth muscle cells that they can participate in the regulation of cell proliferation and that their expression varies during the cell cycle, with less channels expressed in G1 phase while S and M phases are associated with an increased expression [103].

In 2000, Haverstick et al. showed that the blockade of calcium entry in prostate cancer cells (PC-3 and LNCaP) by TH-1177 reduced the proliferation of tumour cells [104]. Interestingly, they showed that injection of TH-1177, in a mouse model of prostate cancer, lengthened the survival of the animals and that no side effects were observed. The mechanism blocked by TH-1177 was mediated by ATP stimulation and might involve a capacitive entry of calcium. Later on, it has been shown that the capacitive entry of calcium was mediated by a T-type calcium channel [105]. These authors clearly showed that the isoform involved was the Cav-3.2 (or α1J) or its splice variant δ25B. The Cav-3.2 was found in Jurkat cells (leukaemic cells), PC-3 and DU-145 (prostate cancer cells) and MDA-MB-435 (breast cancer cells) while the splice variant δ25B was found in PC-3 (prostate and cardiac cells) and MDA-MB-231 and MDA-MB-361 (breast cancer cells). The role of Cav-3.2 and its splice variant δ25B in breast cancer has been reinforced recently in a study by another group where the authors searched for mutations in breast cancer compared to adjacent normal tissues. They found a mutation upstream from the Cav-3.2 locus with abnormal expression of Cav-3.2 in breast cancer tissue in Japanese women [106]. Thus it is clear that Cav-3.2 is over-expressed in breast cancer and as such has been proposed to be a marker [106,107].

The involvemnt of Cav-3,2, a VGCaC, in the capacitive entry of calcium is surprising since this process is normally voltage-independent. An explanation could be brought by the work of Mariot et al. [108] in LNCaP prostate cancer cells. They showed that Cav-3.2 channels are responsible for a calcium entry into the cells, which facilitates neurite lengthening (associated with the occurrence of a neuroendocrine phenotype, and thus more aggressive tumours). This calcium entry is not due to variations of the membrane potential leading to the opening of VGCaC, but to the "window current" due to the fraction of opened VGCaC at the membrane potential of the cells. Thus, it is possible that the neo-expression of Cav-3.2 in cancer cells might lead to an increased entry of calcium, sensitive to external factors such as ATP. This phenomenon can potentiate some of the effects due to the activity of potassium channels (see the paragraph on Potassium channels). This enhanced calcium entry might increase cell proliferation and tumour aggressiveness by means which have to be determined (regulation of proteases? regulation of gene expression?).

The discovery of the role played by Cav-3,2 in the proliferation of tumour cells led some groups to start programs dedicated to develop new blockers of these channels in order to inhibit tumour proliferation [107,109]. Gray and MacDonald [107] developed new antibodies directed toward the Cav-3.2 isoform and its splice variant δ25B. These antibodies present the interest to block the activity of the channels and can be associated with other calcium blockers to treat epithelial cancers [107].

SODIUM CHANNELS

Voltage-gated sodium channels (VGNaC) are classically described as critical elements of action potential initiation and propagation in excitable cells. However, these channels are also known to be expressed in non excitable cells like T-lymphocytes where their role is not elucidated [110]. Sodium channels are transmembrane proteins made of a main α subunit associated with at least two β auxiliary subunits [111]. Ten genes encoding α subunits have been identified and nine of these have been functionally studied in expression systems. Among the ten isoforms, nine constitute a single family named NaV1 according to their phylogeny and are designated NaV1.1 to NaV1.9 [8,112]. The nine isoforms are divided in two classes according to their sensitivity to tetrodotoxine (TTX). TTX-sensitive channels (TTX-S) are inhibited by nanomolar concentrations of TTX (NaV1.1 to NaV1.4, NaV1.6 and NaV1.7) while TTX-resistant channels (TTX-R) are blocked by micromolar and even greater concentrations of TTX (NaV1.5, NaV1.8 and NaV1.9). TTX has not been reported to affect targets other than VGNaC and as such is a pharmacological tool of choice.

The first description of VGNaC in cancer cells was made in 1995 in a rat prostate cancer cell line [113]. Later on, a similar channel has been observed in a human prostate cancer cell line [114]. Molecular biology and pharmacological studies have shown that the main isoform expressed is the NaV1.7. Thanks to its TTX sensitivity, the biological consequences of the abnormal ion channel activity have been addressed. It has been shown that active VGNaC participates in the galvanotaxis (ability of cells to respond to an electric field by moving directionally), motility and invasive potential of these cancer cells [115-117]. Importantly, this abnormal expression of VGNaC in cancer cells has been observed in tumour specimens at the protein and mRNA level [118,119]. Interestingly, the group of Djouad found out that the isoform expressed in prostate cancer is a foetal variant of the NaV1.7 (fNaV1.7) [120]. Thus the expression of fNaV1.7 has been proposed to be a potential novel marker for human prostate cancer and a novel chemotherapeutic target [121].

These results obtained in prostate cancer cell lines led some research laboratories to develop new molecules targeted against VGNaC in order to block its activity [122].

To explain the involvement of such ion channels in migration and invasive properties of the cells, two main hypotheses are put forward beside specific prostate specificities linked to the citrate metabolism [7]. The first one involves the acquisition by the epithelial cancer cells of the ability to produce action potential [123]. This hypothesis is based on the observation that prostate cancer cells may acquire a neuroendocrine phenotype which is a sign of an increased invasiveness [108] even if it has not been possible, up to now, to observe or trigger an action potential in these cells. The second hypothesis is based on the observation that, at the membrane potential of the cells, there is a continuous entry of sodium into the cells [7,124] as it has been observed for Cav-3.2 channels (see the paragraph on Calcium channels). This entry of sodium might alter the homeostasis of calcium and proton which in turn can modify the regulation of proteases through changes in the excretion and/or the activity of the enzymes. These enzymes might facilitate the migration of cells to through the regulation of the actin cytoskeleton or through the digestion of the extracellular matrix [7].

VGNaC have since been found in metastatic cell lines and specimens from tissues other than prostate. They have been described in the breast cancer cell line MDA-MB-231 [124,125] and in breast cancer specimen [125], in the small-cell lung cancer cell lines H146, H128 and H69 [126,127], in the non-small cell lung cancer cell lines Calu-1, H23, H460 and in lung cancer
specimen [7,128], in the leukaemia cancer cells K562, CCRF-CEM and Jurkat [129-131] and more recently in primary cultures of human cervical cancer [132] and of malignant pleural mesothelioma [133]. Several of these studies have assessed the activity of NaV and have shown that it participates in the invasiveness of the cells in vitro. However, the isoform of NaV expressed depends on the localization of the primary tumour. NaV1.7 is the main isoform found in prostate cancer while in breast cancer it is NaV1.5. For the other cancers, the determination of the isoforms has not been performed. In cervical cancer cells, the VGNaC expressed are TTX-S but normal cells already express the TTX-S isoform, NaV1.4. Thus it is unclear whether, with the development of the cancer phenotype, there is an up-regulation of an existing channel or if new isoforms are expressed (mainly NaV1.7?). Although the isoform of VGNaC expressed in neoplastic mesothelial cells has not been determined precisely, it clearly is TTX-S. For non-small cell lung cancer, the situation is rather fuzzy. Indeed, depending on the cell lines, we can observe functional channels belonging to the TTX-S and/or to the TTX-R [in that case it seems that the NaV1.5 is the best candidate] [128]. This discrepancy has also been observed when cancer and associated non-cancer lung biopsies collected from the same patients were assessed for the mRNA coding for VGNaC [7]. What is clear for lung cancer, from the cell line study, is that the interest must not be put on the mRNA but on the proteins. Indeed, we found that all cell lines, even those lacking the sodium current, expressed mRNA encoding for VGNaC. At the level of the protein, we found that cell lines with functional channels display only one band at 250 kDa in western blot experiments while those lacking the sodium current showed two bands: 230 and 250 kDa. These results, which must be confirmed and extended to other cancer tissues, underline the interest which must be put on the protein detection more than on the mRNA. Indeed, the two pathways linked to that topic are antibodies-based detection kits of potentially metastatic cancers [121,134].

Furthermore, it should be noted that for the two cancers in which the determination has been done, the isoform which is expressed is always a foetal splice variant. This particularity might explain why, in breast cancer cell line MDA-MB-231 which expresses NaV1.5, an inhibition of the sodium current is observed with verapamil and diltiazem at concentrations known to block L-type calcium channels, their "natural and clinical" targets [135]. Also, Fraser et al. [125] exploited this difference to produce antibodies which can distinguish between the adult (cardiac) and the foetal (breast cancer) isoform to detect and block the activity of the latter, not of the former [120].

It is of interest to notice that some dietary habits have been shown to protect against cancer. This is the case of breast cancers which are known to be partly prevented by a fish-enriched diet [136]. It is quite admitted, although their mechanisms of action are not clearly delineated, that long-chain omega-3 poly-unsaturated fatty acids play a role in the prevention of cancer. A lot of hypotheses have been put forward to explain such a beneficial effect. By comparison with what is known for the protective role of omega-3 fatty acids in heart diseases, Judé et al. [137] proposed that ion channels like NaV1.5 could be the targets of the dietary lipids and could explain their protective effects. Later on, Isbilen et al. [138] partially confirmed that hypothesis in a breast cancer cell line. However, other experiments are needed to determine the role played by omega-3 fatty acids on NaV in their general beneficial effects.

In the same frame of mind, TTX is proposed to be used in cancer treatment but mainly for its analgesic properties [139]. But it has also been observed that globefish extract was able to cure the disease. It is postulated in this patent that if TTX stops pain this is the other compounds present in the extract which are the active molecules against cancer [140].

CHLORIDE CHANNELS

In 1999, Gruber and Pauli showed that in tumourigenic breast cancer cell lines [MDA-MB-231, MDA-MB-435, MCF-7], a calcium-activated chloride channel (CLCA2) was down-regulated [141]. Stable reintroduction of CLCA2 in these cell lines reduced Matrigel® invasion in vitro and metastatic tumours of MDA-MB-231 cells in nude mice. This finding led to the proposal that calcium-activated channels CLCA2 may act as tumour suppressors in breast cancer. Later on, CLCA1 and CLCA2 were found to also be tumour suppressors in human colorectal cancer [142]. After these papers, a lot of works were performed to understand how a chloride channel could be tumour suppressor. However, the "channel" properties of the CLCA family proteins were questioned. Indeed, depending on the model used, CLCA2 might have either five or one transmembrane segments. In 2006, the lab of Gruber and Pauli showed that CLCA2 had a single transmembrane segment and as such could not be an ion channel [143].

No other chloride channel has been involved to our knowledge.

REGULATION OF ION CHANNELS BY SIGMA RECEPTOR

Sigma receptors are intracellular proteins that were first postulated as opioid receptors on the basis of pharmacological and behavioural studies [144]. Finally, 30 years of pharmacological works have revealed the existence of two different "sigma binding sites" named sigma-1 and sigma-2 receptors [145]. While the sigma-2 receptor has not yet been identified, the cloning of the sigma-1 receptor subtype in 1996 had surprisingly revealed a 29 kDa protein putatively containing two transmembrane domains [146]. The gene is located on chromosomes 9 and 2 in human and rodents, respectively. The protein is composed of 223 amino acids with 87-92% identity and 90-93% homology among tissues and species. Surprisingly, the sequence shares no homology with classical mammalian receptor sequences, but rather a 33% identity and 66% homology with a yeast sterol isomerase and with emopamil binding protein [147]. However, if the sigma-1 receptor presents a steroid-binding domain, the protein has no sterol-isomerase activity [146,147]. The sigma-1 receptor is localised at the inner face of the plasma membrane, and the membranes of the endoplasmic reticulum and the nucleus [146-150]. While high concentrations of neurosteroids (DHEA, pregnenolone and progesterone for example) have been shown to interact with brain sigma-1 receptors in behavioural studies [151], no high-affinity endogene sigma receptor ligand has yet been identified. Nonetheless, exogenous compounds from disparate chemical classes ((+)-benzomorphans, cocaine, guanidines and neuroleptics for example) have been characterized or developed as highly selective sigma receptor ligands [152].

Sigma receptors are expressed in various tissues including discrete areas in brain, pituitary, kidney, testis, ovary and liver [147,153-155]. Surprisingly, very high levels of sigma receptors have been detected in tumour cells when compared to normal cells [156,157]. The presence of both sigma-1 and sigma-2 receptors as been revealed in breast, lung (SCLC and NSCLC), brain, colon, prostate and blood cancer [150,156,157]. Cells lines derived from lung, breast and prostate have been compared with normal cells of the same origin. The level of sigma-1 receptor mRNA was 4- to 20-fold higher in tumour cell lines than in normal cells. Moreover, the expression of the sigma-1 receptor seems to be dependent on the phase of the cell cycle: a maximal level was detected in the M phase whereas a minimal level was reached at the G1/S transition [158]. In cancer biopsies, the expression of sigma receptors is correlated with the proliferating state of the cells so that these proteins are now commonly considered as tumour bio-markers [159,160]. For example, sigma-1 receptors have been shown to represent interesting prognostic factors in breast cancer [159]. Consequently, during the past 15 years, many chemical compounds...
presenting a sigma receptor affinity have been developed for tumour imagery (PET scan) in order to detect tumours at an early stage [161,162]. However, if sigma receptors represent exciting targets in order to detect cancers in vivo, very few data are available on the function linked to these mysterious proteins in tumour cells. One of the most exciting challenges is to understand the cellular mechanisms coupled to sigma receptors: early in vitro studies had revealed that incubation of tumour cells with exogenous sigma receptor ligands provoked an arrest of cancer cell growth. Brent & Pang showed that in 6 tumour cell lines (adenocarcinoma, colon mammary carcinoma, and melanoma), sigma-1 and sigma-2 receptors ligands diminished the cell number after 2 days incubation [163]. The same results have been recently obtained with mammary carcinoma, prostate cancer and SCLC cells [156,164,165]. In a recent series of studies by Sanofi researchers, a sigma-1 receptor ligand currently in a clinical trial, SR31747A, has been shown to inhibit tumour cell growth both in vivo and in vitro [166]. However, despite their real interest, the descriptive character of these studies let unsolved important questions such as: Which are the transduction pathways associated with sigma receptors? Do sigma receptors increase the growth rate of tumours by preventing cell cycle arrest triggered by errors detected at checkpoints or by the inhibition of defence mechanisms leading to programmed cell death?

Recently, a significant break through for the understanding of the role of sigma receptors in tumours has been accomplished by the demonstration of a functional interaction between sigma receptors and membrane ion channels.

**Sigma-1 Receptors Modulate K⁺ Channels in Tumour Cells**

In the late 90’s, a first clue has been put forward by demonstrating a clear functional relationship between sigma-1 receptors and the bioelectrical activity of neuroendocrine cells. Using the model of frog pituitary melanotrope cells, Soriani et al. demonstrated that the application of highly selective sigma-1 ligands such as pentazocine or igsine, enhanced the firing of action potentials, leading to a stimulation of hormone secretion [167]. This modulation was indeed the consequence of the inhibition of various voltage-operated K⁺ channels (VOKC) subtypes including the delayed rectifier [167], the transient outward [168] and the M-current [154]. These results were next confirmed in rat pituitary [169]. However, while the results obtained with frog pituitary indicated a coupling involving a Gs protein [167,168], the mechanism rather appeared to be a direct protein/protein interaction between sigma-1 receptor and K⁺ channels in rat pituitary [170]. This discrepancy may be explained by two differences: the species and the pituitary models used. The study was performed at the postsynaptic level in frog pituitary whereas it was done at the presynaptic level in the rat model. These data reveal the variety of coupling mechanisms that actually exist among cell types between sigma receptor and the molecular targets. The work of Soriani and co-workers then focused on tumour cells. They demonstrated both in SCLC cells and in leukaemic cells that the coupling is functional between sigma receptors and VOKC. More interestingly, either sigma receptor ligands or specific VOKC blockers arrested cell growth through a common pathway. These results revealed for the first time that sigma receptor ligands and VOKC blockers induced a G1 phase cell cycle arrest involving the accumulation of the cyclin inhibitor p27kip1 and the subsequent reduction in the expression of cyclin A. This pattern is a common signature of an arrest in the G1 phase because cyclin A is necessary for the cell to progress in S phase [171].

By which kind of mechanism can K⁺ channel activity alter cell cycle? While it is now widely accepted that these channels are strongly involved in the control of cell division, this exciting question still remains unanswered [14]. Among the various hypotheses proposed, a mechanism involving the regulatory volume decrease (RVD) is worth being looked at. RVD is a cellular response to osmotic stress shared by all animal cells, allowing them to recover a normal volume after a membrane swelling through the loss of water and KCl. This process is tightly regulated by potassium and chloride channels. The molecular nature of the channels involved varies among cell types for K⁺ channels and remains unclear for VRCC [172,173]. Interestingly, the RVD process has been shown to participate in the control of cell cycle progression because its inactivation blocks cells at the end of the G1 phase [174,175]. A cycling cell encounters osmotic stresses that are the consequences of the accumulation of metabolites in the cytoplasm or the depolymerisation of the actin cytoskeleton, both leading to a cell swelling. It has been proposed that the cell volume is a key factor that is controlled at the end of the G1 phase to allow the transition toward the S phase (volume set point). A constant volume may help maintaining the correct concentrations of cell cycle-controlling proteins; a well regulated volume may also avoid the destabilisation of the cytoskeleton in response to membrane stretches that may in turn alter the ROCK/mDia balance and thus lead to p27kip1 accumulation and the subsequent arrest of the cell cycle [176]. As a matter of fact, the inhibition of K⁺ channels by sigma receptor ligands in both leukaemic and SCLC cells may impair the RVD process, leading to the arrest of cell growth we have described [171]. This hypothesis has been checked recently by Renaudo et al., [150]. It was found that incubation of tumour cells with sigma receptor ligands strongly inhibited the RVD. In the course of that study, it was also demonstrated that sigma receptor interacted with VRCC which are the counterpart of K⁺ channels in the modulation of RVD. Similar to what was observed with K⁺ channels blockers and sigma receptor ligands, VRCC blockers arrested the cell cycle in the G1 phase and led to the accumulation of p27kip1 and the decrease in cyclin A content in both SCLC and jurkat cells, supporting the existence of a common pathway [150].

Altogether, these results allow proposing a first scheme to explain the effects of sigma receptor ligands on the growth of tumour cells: the inhibition of K⁺ and Cl- channels provoked by the pharmacological activation of sigma-1 receptors stops the cell cycle at the G1 phase through an inhibition of the RVD [150].

However, at this stage, a question still arises from these observations. Why do tumour cells over-express sigma-1 receptors? A striking clue came from the thorough analysis of the kinetic properties of the VRCC in HEK cells stably transfected with the sigma-1 receptor. This model (HEK-SIG cells) is valuable to make comparative studies because wild-type HEK cells express very low levels of sigma-1 receptors in normal conditions. While the expression of the sigma-1 receptors did not alter the current density, it was able to provoke a huge delay in the kinetics of activation of the current in the absence of any exogenous ligand [150]. It has also been reported that the RVD process is not inhibited but delayed in HEK-SIG cells when compared to control cells.

In which way would a delayed VRCC be an advantage for tumour cells? Recent studies have nicely demonstrated that the cell shrinkage occurring during apoptosis (apoptotic volume decrease or AVD) was an early stage of the signalling cascade in various tumour cell types [177,178]. This volume-related signalling event is linked to the activation of both potassium and VRCC currents, and the subsequent KCl-coupled water efflux [177-179]. In the light of these data, Renaudo et al. proposed that the strong expression of the
Sigma-1 receptors increases the apoptosis resistance in tumour cells through the down-regulation of the VRCC activation. In support of this, they have demonstrated that the staurosporin-triggered AVD was diminished in HEK cells expressing sigma-1 receptor when compared to wild-type HEK [150]. This hypothesis was reinforced by a recent study showing that cells expressing sigma-1 receptor, such as tumour cells, are less resistant to apoptotic stress than wild-type cells, and that this resistance is abolished by sigma receptor antagonists [180]. Moreover, Aydar et al. [146] have recently demonstrated that the expression of sigma-1 receptors was able to produce a tonic down-modulation of voltage-dependent potassium channels in the absence of exogenous ligand in Xenopus oocytes, suggesting that potassium channels also participate in the sigma-1 receptor-dependent apoptosis resistance we propose here. In conclusion, these studies demonstrate for the first time that sigma-1 receptors, through the interaction with and modulation of chloride/potassium channels, lead to an impairment of cell volume regulation. In the absence of exogenous sigma receptor ligands, the over-expression of sigma-1 receptors limits VRCC activity so that the protection against AVD is reinforced but not enough to block RVD and cell cycle progression. In contrast, when tumour cells are challenged with sigma receptor ligands, this balance is strongly altered and the RVD is inhibited, leading to an arrest of proliferation (Fig 4).

A recent work has demonstrated that sigma-1 receptors modulate calcium-dependent K⁺ channels of the SK family in brain [181]. Because these K⁺ channels has been shown to interact with the migration process of tumour cells [4], the interaction between sigma-1 receptors and ion channels in tumours may not be limited to cell division or apoptosis aspects, but could also regulate invasiveness of cancer cells.

Altogether, these data pave a new avenue in the comprehension of both function and action mechanisms of sigma-1 receptors and may lead to the development of ion channel-targeted cancer therapy through the modulation of sigma-1 receptors function.

**Sigma Receptors Interact with Ca²⁺ Channels**

The effects of sigma receptor ligands on Ca²⁺ channels expressed in tumours have never been studied so far. Some data can be found from other tissues; it has been previously shown that sigma-1 receptor ligands such as (+)-pentazocine were able to enhance voltage-activated Ca²⁺ channels in neuroendocrine and neuroblastoma cells [154, 182]. In contrast, Zhang et al. have recently described the inhibition of N and L, P/Q and R voltage-dependent Ca²⁺ channels through sigma-2 receptors [183]. Altogether, these results suggest that sigma receptors interact with VOC in tumour cells such as neuroblastoma or SCLC cells which express voltage-activated calcium channels. Further studies would be necessary to clarify this point.

**Patents in the Field of Sigma Receptors Ligands and Cancer**

The number of patents in the field of cancer and sigma receptors is rather low and mainly concerns imagery (PET scan) for the early detection of tumours. Following studies which demonstrate that the expression of sigma-2 receptors is linked to the quiescent versus proliferative status of cells [160,184,185], several sigma-2 receptor selective ligands, including benzamides, have been patented [186-188]. Some of these patents extend the use of sigma receptor ligands to anti-proliferative treatment [186-188]. Interestingly, a patent proposed the sigma-1 antagonists rimecaze and IPAG for cancer treatment. The patent indicates that these compounds inhibit endothelium cell proliferation, thus limiting angiogenesis [189]. More recently, 3,11b-cis-dihydrotetrabenazine, a sigma-1 receptor ligand, has been patented for the treatment of inflammatory and proliferative diseases [190]. No patent related to the interaction between sigma receptors and ion channels in cancer has yet been proposed to our knowledge. Consequently, the studies in this domain will eventually allow proposing new targets to modulate tumour ion channels through sigma receptors and will provide better understanding for the mechanisms of action of already patented sigma receptor ligands.

**CURRENT & FUTURE DEVELOPMENTS**

The finding of new targets involved in cancer to better cure the disease is a great challenge for the next few years. The role of ion channels in the different aspects of the diseases is beginning to emerge: for example some potassium and calcium channels in proliferation, some others in migration; sodium channels in migration and invasion... As such, ion channels open a completely new field of investigation. This opening field started to be explored by some researchers who patented potassium channels as potent markers and targets of therapies [191-200]. Up to now, no chemo-

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**Fig. (4). Function of sigma-1 receptors in tumour cells.** The sigma-1 receptor is over-expressed in tumour cells. It results in a down-regulation of the basal activity of voltage-activated K⁺ channels and volume-regulated Cl⁻ channels involved in volume regulatory process. The tumour-specific over-expression of sigma-1 receptors protect cells from apoptosis by delaying the shrink (Apoptosis Volume Decrease, AVD) occurring upon apoptotic stress. While this tonic inhibition of ion channels does not alter the cell division cycle, the pharmaceutical activation of sigma-1 receptors by exogenous sigma ligands enhances the inhibition of K⁺ and Cl⁻ channels. Consequently, the RVD necessary during cell cycle is strongly altered so that the G1/S volume set point can not be passed.
therapy has intentionally been designed to inhibit ion channels even if we cannot rule out the possibility that some of the existing chemotherapeutic drugs are beneficial to the patient thanks to "ion channel-blocking" side effects. This can be the case for TTX which is thought to relieve pain by blocking Nav and as such can participate in the treatment itself [139].

Different ways can be explored to exploit this novelty. First, ion channels can be used as early event markers in the process of oncogenesis. For example, it has recently been proposed that TRPM8, a non voltage-dependent calcium permeable channel, is a highly-specific prostate and prostate cancer-associated marker which could be used as a potential target for therapies [201-203].

It has recently been shown that the expression of Cα3.2 is related with the development of breast cancer [106]. In a similar way, Fraser et al. [125] suggested that the presence of mRNA coding for fNav1.5 in the primary tumour could be an early marker of metastases, which could therefore be predicted before the invasion of the lymph-node [120]. It is not foolish to propose that other channels, probably potassium channels like hEAG, hIKCA, GIRK1 and SK3, thanks to their early detection, could help to cure patients before the burst of the disease.

Also, ion channels can be targeted to stop the oncogenesis or prevent the process of metastasis, To do that, new molecules must be developed. Some of the channels abnormally expressed in cancer can be normally expressed in the brain or the heart. So a great care must be taken to selectively affect ion channels in cancer cells and not in cardiac cells. This is the case for Nav1.5 which is the cardiac isoform of sodium channels but which can be expressed in some breast cancers. How will it be possible to distinguish between both tissues? Some strategies can be based on antibodies recognizing isoforms specific of the tumour tissues. In such a way, Chioni et al. [204] developed an antibody targeted against the foetal isoform of Nav1.5. This antibody (NESOaPb) blocks the ion channel of cancer cells. It does not recognize the adult variant expressed in the heart and is therefore likely to have fewer cardiac side effects when used as a therapeutic drug.

Another strategy is to develop new organic molecules on the basis of their selectivity against the channel and of their lesser cytotoxic effect against normal cells. This is the strategy used by the group of Robert Sikes in Newark (USA) and Philippe Bougnoux in Tours (France). This strategy is beginning to be rewarding since the first molecules have been patented [102].

To summarize, the emerging place of ion channels as new early markers of oncogenic events such as metastasis and as new targets of anti-oncogenic drugs opens new fields in cancer therapy.

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REFERENCES


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[61] Lu K, Means A. Regulation of the cell cycle by calcium and calmodulin.


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Djamgoz M, Fraser, S., Diss, J. WO0218637 (2002).


Su TP, Hayashi T. Understanding the Molecular Mechanism of Sigma-1 Receptors: Towards A Hypothesis that Sigma-1 Receptors are Intracellular Amplifiers for Signal Transduction. Curr Med Chem 2003; 10(20): 2073-80.


Hayashi T, Su TP. Sigma-1 receptor ligands: potential in the treatment of neuropsychiatric diseases. CNS Drugs 2004; 18(5): 269-84.


Zamora PO, Moody TW, John CS. Increased binding to sigma sites of [1-(2-piperidinyl)ethyl]-4-[1-(25)-sodobenzamide (1-(25)-PAB) with onset of tumor cell proliferation. Life Sci 1998; 63(18): 1611-18.


