

K⁺ Channels and Cancer:

Surprising New Discoveries

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K⁺ channels belong to a large class of transmembrane proteins that specifically regulate the transport of K⁺ ions across biological membranes. Today about 80 different K⁺ channels have been identified which are expressed in virtually every cell type. K⁺ channels are involved in a wide variety of physiological functions including neurotransmitter release, regulation of heart rate, insulin secretion, epithelial electrolyte transport and immune function. Moreover, an increasing number of hereditary human diseases can be linked to K⁺ channel dysfunctions including epilepsy¹, cardiac arrhythmias², skeletal muscle disorders³ and diabetes.⁴ In addition, mounting evidence demonstrates that K⁺ channels are involved in cell proliferation and cancer progression. In this review we will summarize the latest findings concerning K⁺ channel involvement in cancer development.

Introduction

K⁺ channels are the largest ion channel family in the human genome comprising about 80 different gene products. They can be structurally divided into three subfamilies: the voltage and Ca²⁺-dependent K⁺ channels (K_v and K_{ca}), the inward rectifying K⁺ channels (K_{ir}) and the two-pore K⁺ channels (K_{2p}).⁵

The K_v family is the largest of the three and as its name implies, the channels open in response to a voltage change (or depolarization) of the cell membrane, thus allowing an efflux of K⁺ ions. K_{ca} channels open in response to a rise in intracellular Ca²⁺ and similarly permit the selective efflux of K⁺ ions from the cell. The functional K_v and K_{ca} channels are composed of four six-transmembrane (TM) α subunits arranged surrounding a central ion-conducting pore. The K_{ca}1.1 (also known as the BK_{ca} or Maxi-K channel) is unique in that it can respond to both membrane depolarization and a rise in Ca²⁺ and has an extra TM domain making it the only K⁺ channel with seven TM domains.

The inward rectifying K_{ir} channels derive their name from their capacity to conduct K⁺ ions inward rather than outward. K_{ir} channels activity is modulated by a remarkable array of intracellular second messengers including phosphatidylinositol bisphosphate (PIP₂), G protein $\beta\gamma$ subunits and ATP. The K_{ir} channels have the simplest topology of the K⁺ channel

superfamily with only two TM domains flanking the pore loop. As is the case with the K_v channels, four subunits are needed for assembly into a functional channel.

The two-pore K_{2p} channels are composed of four TM domains with two pore loops and hence their name. These channels are sometimes called “leak” or “background” channels, as they are constantly open at the physiological membrane potential. They can be modulated by various agents including changes in temperature and pH and mechanical stress.

As mentioned above, an increasing body of work shows that tumor cells express a diverse array of K⁺ channels and that these channels may play a role in the regulation of tumor proliferation, migration and apoptosis (see the comprehensive recent reviews in references 6-9). In this review we will summarize the most up to date information regarding the involvement of several K⁺ channels in tumor progression.

A. K_v10.1 (*eag1*) and K_v11.1 (HERG)

The K_v10.1 and the K_v11.1 are voltage-gated K⁺ channels that belong to the same channel family: the *ether-a-go-go* (EAG) family. The EAG family of voltage-gated K⁺ channels can be subdivided into three distinct groups based on sequence homology. They are the *eag* (K_v10) with two

members, the *eag*-related channels (*erg* or K_v11) and the *eag*-like K⁺ channels (*elk* or K_v12) with three members each.

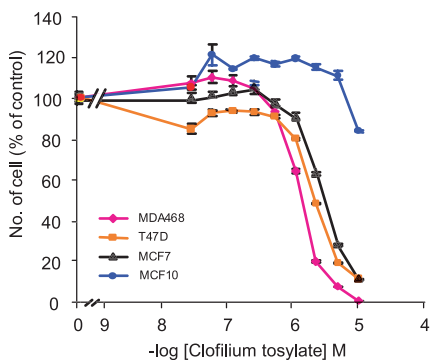
The K_v11.1 (*erg1*) channel has received considerable attention since it underlies the *I_{Kr}* current in the heart and has a central role in mediating repolarization of cardiac action potentials. Mutations in the K_v11.1 channel cause inherited long QT syndrome (LQTS) that is associated with life-threatening arrhythmias and sudden death.¹⁰ Moreover, drug-induced forms of LQTS have been reported for a wide range of non-cardiac drugs including antihistamines, psychoactive agents and antimicrobials. All these drugs potently block the K_v11.1 channel as an unintended side effect, prompting regulatory drug agencies to issue recommendations for the testing of new drugs for their potential K_v11.1 blocking effect.

In the last few years, a growing number of studies have shown that K_v11.1 is selectively upregulated in a variety of human and animal tumors while its expression is absent in the normal tissue or cell line counterparts.¹¹⁻¹⁷ Moreover, selective pharmacological blockage of the K_v11.1 channel in several primary leukemic cells significantly reduced cell proliferation,¹³ while in colon cancer cell lines it blocked cell invasiveness.¹⁷

It is still unclear, however, how overexpression of the K_v11.1 channel contributes to the neoplastic phenotype. One possibility is that the special

biophysical properties of $K_v11.1$ channels contribute to maintain a more depolarized membrane potential (a characteristic of tumor cells) and thus permit an easier passage through the cell cycle. Another mechanism that could explain $K_v11.1$ overexpression function in tumor development is through its interactions with several proteins involved in proliferation

Inhibition of Breast Cancer Cell Lines Proliferation by a K^+ Channel Blocker



Clofilium tosylate a non specific K^+ channel blocker inhibits growth of breast cancer cell lines (MCF-7, MDA-468, T-47D) but not normal breast cell line (MCF-10).

control. In a recent report, it was showed that the oncogene *v-src* (a constitutively active form of the protein tyrosine kinase *src*) could phosphorylate the $K_v11.1$ channel and thus induce an increased current.¹⁸ Since aberrant function of proteins in the *ras-src* signaling pathway is a common feature of transformed cells, *src*-mediated modulation could be a mechanism that regulates $K_v11.1$ function in cancer cells. Another study showed that the $K_v11.1$ channel expressed in cell lines and primary tumors was preferentially a heterotetramer formed by the “regular” $K_v11.1$ gene transcript and an alternative splice variant termed $K_v11.1-b$ (*herg1b*). The biophysical properties of the resulting channel, turned out to be quite different than those exhibited by $K_v11.1$ in normal cells. In addition, the expression of the two $K_v11.1$ protein isoforms was strongly cell cycle-dependent.¹⁶

Another study demonstrated that $K_v11.1$ protein physically interacted with the tumor necrosis factor receptor type 1 (TNFR1) in the cell membrane of tumor cell lines.¹⁵ TNFR1 is the ubiquitously expressed receptor for the TNF α cytokine that can mediate both cell proliferation and apoptosis in many cells. The significance of its interaction with the $K_v11.1$ channel however, is not clear.

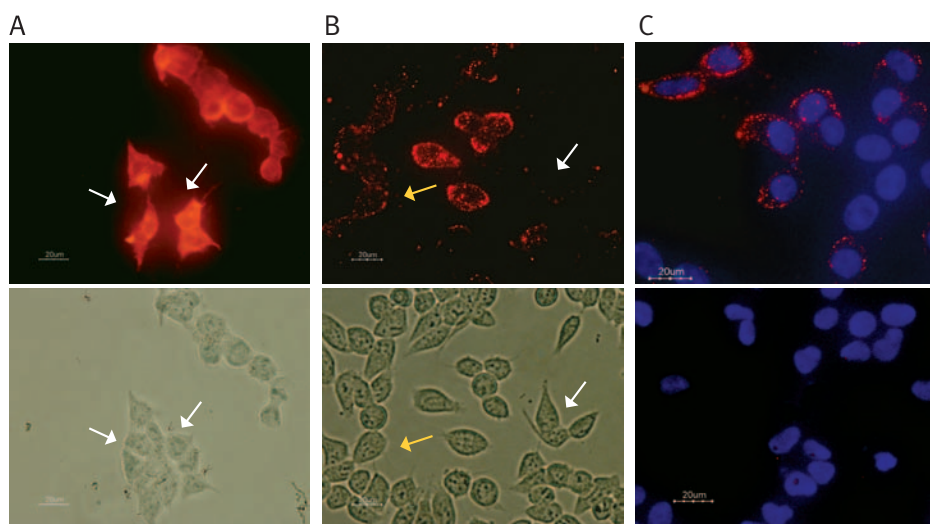
In addition, $K_v11.1$ was found to interact with the

adaptor protein 14-3-3, a family of conserved proteins involved in the control of cell cycle regulation, apoptosis and adhesion.¹⁹

Finally, $K_v11.1$ was shown to physically interact with the integrin $\beta 1$ protein and modulate adhesion-dependent integrin signaling.²⁰

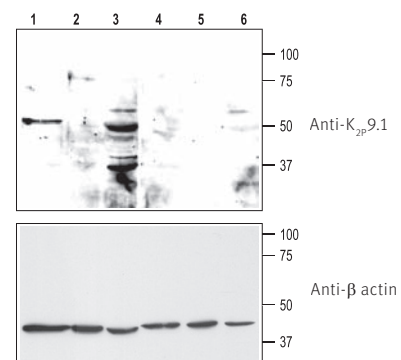
Another K_v channel that has been directly implicated in malignant transformation is the $K_v10.1$ channel. The $K_v10.1$ channel current is characterized by outward rectification without inactivation and slow activation kinetics. Its expression is normally confined to the brain where its physiological function has not yet been clarified. However, several groups found evidence indicating that $K_v10.1$ was inappropriately expressed in several cancer cell lines and in human primary tumors.²¹⁻²³ Moreover, one study showed that $K_v10.1$ by itself had oncogenic potential as a cell line transfected with the channel induced aggressive and faster tumor growth *in vivo* as compared to a cell line transfected with an unrelated K_v channel.²² The same study also showed that inhibition of $K_v10.1$ expression with antisense oligonucleotides was sufficient to decrease the proliferation of various cancer cell lines. As is the case for the $K_v11.1$ channel, the contribution of $K_v10.1$ to tumor development is believed to be related to its ability to modulate cell cycle progression.

Cell Surface Expression of the $K_v11.1$ K^+ Channel



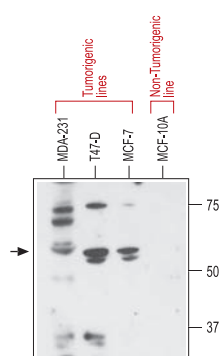
(A) Human embryonic kidney (HEK) cells transfected with the native $K_v11.1$ channel were fixed, permeabilized and stained with an Anti- $K_v11.1$ antibody (#APC-062) that recognizes an epitope situated at the cytoplasmic C-terminus end of the channel. The antibody was visualized with a goat-anti-rabbit Alexa-555 secondary antibody (in red-upper panel). Note that almost all cells are positively stained. White arrows indicate cells that do not express the $K_v11.1$ channel. (B) Staining of live intact HEK- $K_v11.1$ cells with Anti- $K_v11.1$ -extracellular antibody (#APC-109) followed by goat-anti-rabbit Alexa-555 secondary antibody. About half of the cells show little (yellow arrows) or no staining (white arrows) while others have strong staining. Indicating that surface expression of the channel does not fully correlate with total cell channel expression (as seen in (A)). (C) Specificity of the Anti- $K_v11.1$ -extracellular antibody was confirmed by incubating intact living HEK- $K_v11.1$ cells with the antibody (upper panel) or with the antibody preincubated with the peptide antigen (bottom panel), followed by goat-anti-rabbit Alexa-555 secondary antibody (red staining). Cell nuclei were stained with the cell permeable dye Hoechst 33342 (blue staining).

Overexpression of $K_{2p}9.1$ K^+ Channel in Tumorigenic Cancer Cell Lines



Proteins, lines 1 and 4 rat cerebellum lysate, lines 2 and 5 MCF-10A human mammary gland (fibrocystic disease) and lines 3 and 6 MCF-7 human mammary gland (adenocarcinoma), were separated in a SDS-PAGE gel and sequentially probed with Anti- $K_{2p}9.1$ antibody (#APC-044) (upper panel) and anti- β actin to ensure equal loading (lower panel). In lines 4, 5 and 6 the antibody was preincubated with the control peptide antigen. Note that the expected 50 kD MW band is present in cerebellum and in the tumorigenic breast cancer cell line (MCF-7) but not in the non-tumorigenic breast cell line (MCF-10A).

Expression of $K_{ir}3.1$ K⁺ Channel in Breast Cancer Cell Lines



Cell lysates from three tumorigenic breast cell lines and one non-tumorigenic were separated in a standard SDS-PAGE and analyzed by Western blotting with an Anti- $K_{ir}3.1$ antibody (#APC-005). Note that the antibody a band of the appropriate size (arrow) in the three tumorigenic lines but not in the non-tumorigenic one.

B. $K_{2p}9.1$ (TASK-3)

The $K_{2p}9.1$ K⁺ channel (also known as TASK-3 or KCNK9) belongs to the two-pore K⁺ channel family. As mentioned above, members of this family show little time or voltage dependence and therefore are continuously open at physiological membrane potentials and thereby help set the resting membrane potential. $K_{2p}9.1$ is expressed at low levels in normal tissues except in the brain where it is widely distributed and its expression overlaps with the structurally related $K_{2p}3.1$ (TASK-1, KCNK3) channel.

The physiological role of the $K_{2p}9.1$ channel is not yet clear, though it was proposed to participate in breathing, aldosterone secretion, anesthetic-mediated neuronal activity and in cerebellar granule neuron apoptosis.^{24, 25}

The $K_{2p}9.1$ gene was found to be amplified in 10% of breast cancer samples while the $K_{2p}9.1$ protein was overexpressed in breast, lung, colon and prostate cancers.²⁶ Moreover, overexpression of $K_{2p}9.1$ in cell lines promotes tumor growth and confers resistance to hypoxia and serum deprivation.²⁶

As is the case with the above mentioned examples, the mechanism by which $K_{2p}9.1$ overexpression exerts its oncogenic properties is not clear, but it requires a functional channel since point mutations in the channel that abrogate its ability to transfer K⁺ ions, eliminates the oncogenic properties of $K_{2p}9.1$.²⁷

C. Other K⁺ Channels

Several other K⁺ channels have been implicated in cancer development although their functional

roles are less established.

The voltage-dependent $K_{v}1.3$ channel has been detected by immunohistochemistry methods in both colon and breast cancer samples while the normal tissue counterparts showed no detectable $K_{v}1.3$ expression.^{28, 29} The $K_{v}1.3$ channel is expressed in normal lymphocytes and macrophages (as well as in the brain) where it has a critical function in regulating Ca²⁺ entry and T cell proliferation.³⁰

$K_{ir}3.1$ (also known as GIRK1) mRNA expression was upregulated in about 30% of invasive breast cancer and in 60% of non-small-cell lung cancer specimens examined. The upregulation of the $K_{ir}3.1$ mRNA was correlated in both cases with poor prognostic outcome.^{31, 32} $K_{ir}3.1$ is a member of the G-protein regulated inward-rectifier K⁺ (GIRK) channel subfamily which is part of the inward-rectifier (K_{ir}) K⁺ channel family. $K_{ir}3.1$ can be activated by neurotransmitters and other factors via the activation of G-protein coupled receptors. Binding of the corresponding ligand (for example acetylcholine or adrenaline) to their respective G-protein receptor induces the dissociation of G α -GTP from the G $\beta\gamma$ dimer. The latter directly binds to $K_{ir}3.1$ and activates the channel. $K_{ir}3.1$ is normally expressed in the brain and heart.³³

Finally, members of the Ca²⁺-dependent K⁺ channel (K_{Ca}) family, the $K_{Ca}1.1$ (also known as Maxi-K or BK_{Ca}) and the $K_{Ca}3.1$ (also known as SK4 or IK_{Ca}) have been implicated in the progression of several cancers including gliomas, melanomas and pancreatic cancer.³⁴⁻³⁶

Conclusions

In the last several years several K⁺ channels have been identified at the molecular level as having a direct involvement in tumor progression. For the most part the molecular mechanism by which ion channels modulate tumor growth is unknown. In any case, a substantial array of toxins and drugs that specifically modulate the activity of K⁺ channels is already available. Therefore it is conceivable that in the near future these or other ion channel-targeted molecules will become a valid addition to the growing arsenal of anti-cancer drugs.

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Related Products

Compound	Product #
K⁺ Channel Antibodies	
Anti- $K_{2p}9.1$ (TASK3)	APC-044
Anti- $K_{ir}3.1$	APC-005
Anti- $K_{v}1.3$	APC-002
Anti- $K_{v}1.3$ -extracellular	APC-101
Anti- $K_{v}1.3$ -extracellular-FITC	APC-101-F
Anti- $K_{v}10.1$ (EAG-1)	APC-104
Anti- $K_{v}11.1$ (erg1)	APC-016
Anti- $K_{v}11.1$ (HERG)	APC-062
Anti-hK _v 11.1 (HERG)-extracellular	APC-109
Anti- $K_{v}11.1$ (HERG)-extracellular-FITC	APC-109-F
Anti- $K_{Ca}1.1$ (1098-1196) (BK _{Ca})	APC-021
Anti- $K_{Ca}1.1$ (1184-1200) (BK _{Ca})	APC-107
Anti- $K_{Ca}3.1$ (SK4)	APC-064
K⁺ Channel Blockers	
rAgitoxin-1	RTA-150
rAgitoxin-2	RTA-420
rAgitoxin-3	RTA-390
rBeKm-1	RTB-470
rCharybdotoxin	RTC-325
rErgtoxoin-1	RTE-450
E-4031	E-500
rIberiotoxin	RTI-400
rMargatoxin	RTM-325
rMaurotoxin	RTM-340
Paxilline	P-450
Penitrem A	P-650
rSlotoxin	RTS-410
Tertiapin	T-250
rTertiapin Q	RTT-170
Verruculogen	V-500
K⁺ Channel Openers	
Isopimaric Acid	I-370
Pimaric Acid (PIMA)	P-270