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Review Article

The Emerging Role of Two-Pore Domain Potassium Channels in Breast Cancer

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Abstract

Potassium ion channels are transmembrane proteins that selectively facilitate ion flow down an electrochemical gradient between intracellular and extracellular environments. There is accumulating evidence which suggest that potassium channel protein activity is important in the pathophysiology of cancer, and associations of the two-pore domain family of potassium channels and breast cancer are currently emerging. The aim of this review is to summarize data on mechanisms of action related to oncogenic properties and examine the role of the two-pore domain family in breast cancer.

Keywords

Breast Cancer; Potassium Channels

Abbreviations

CpG	:	Cytosine-Guanine
ER/PR	:	Estrogen/Progesterone Receptor
HER2	:	Human Epidermal Growth Factor Receptor-2
K+	:	Potassium
Kv	:	Voltage-Gated K+ Channels
KCa	:	Calcium-Activated K+ Channels
Kir	:	Inward-Rectifier K+ Channels
K2p	:	2-Pore Domain K+ Channels
LOM	:	Loss of Methylation
nH	:	non-Hispanic
TN	:	Triple-Negative

Potassium Ion Channel Proteins and Mechanisms of Action in Cancer

There is accumulating evidence which supports the role of several types of ion channels in the pathophysiology of cancer [1,2]. Potassium (K+) channels are transmembrane proteins that selectively facilitate the flow of K+ ions down an electrochemical gradient between intracellular and extracellular environments [3,4]. K+ channels are the most diverse and ubiquitous class of ion channels and control membrane potential, are involved in several physiological functions, and contribute to nerve and cardiac action potentials and neurotransmitter release [1].

K+ channels can be classified according to a combination of stimulus, conductance properties and structural criteria which produces 4 classes: (1) voltage-gated (Kv); (2) calcium-activated (KCa); (3) inward-rectifier (Kir); and (4) 2-pore-domain (K2p) [3,4]. K+ channels typically have a basic conserved subunit structure that consists of 2 transmembrane domains and a hairpin structure, known as the pore-forming loop (P-loop); these subunits associate to form channels and are encoded by 79 genes [5]. Among ion transporters, K+ channels show the highest variability and the most frequently altered expression in many tumor types [4].

K+ channels have essential roles in cell behaviors linked to cancer, and highly proliferating cells display a more positive cell membrane potential (an activity driven by K+ channels) than quiescent cells [6]. Importantly, cell cycle and proliferation, cell migration, invasion and apoptosis are all processes that can be modified by K+ channel expression and potentially offer the opportunity for novel therapeutic manipulation [3,4]. With respect to control of K+ channel gene expression in tumors, changes in the expression levels in tumors can occur at the genomic, transcriptional, post-translational or epigenetic levels, and in some cases a quantitative increase in activity can be explained by upstream changes. In most tumors, the abnormally expressed channel is wild type [3,4]. However, a small clinical study genotyped

22 patients with Aldosterone-Producing Adrenal Adenomas (APAs) and found associated mutations in the pore-forming loop of the inward-rectifier Kir3.4 which is encoded by KCNJ5 [7].

Altered K+ channel expression and/or function occurs in a range of cancer types including breast cancer, and ion channels from each of the four main K+ channel families have been implicated. This evidence is summarized below except for K2p genes which is addressed separately in a following section.Recent publications by Pardo and Stuhmer and Comes et al., provide a more in-depth review of the potential roles of K+ channels in cancer [1,2].

Enhancement of tumor cell migration

Kv10.1 (a.k.a. Human ether à-gogo, HERG1) has been found to regulate MDA-MB-231 breast cancer cell migration in a laboratory study on cell lines [8]. Additional cell line studies suggest that Kv11.1 is also implicated in the migration of leukemia, melanoma and thyroid cancer cells [9-12]. Malignant neural cell studies have also shown that KCa1.1 and KCa3.1 appear to be important for the migration of glioma cells [13-17]. Furthermore, a small conductance subfamily member KCa2.3 (or SK3) appears to mediate migration and metastasis of malignant colon, breast cancer and melanoma cells [18-21].

Involvement of tumor proliferation and apoptosis

Kv10.1 overexpression has been implicated in malignant transformation and proliferation in several tumor cell lines including neuroblastoma [22]. Another laboratory study suggested that Kv10.1 also plays a role in controlling the proliferation and/or cell cycle of MCF-7 breast cancer cells [23]. The initiation of apoptosis appears to be associated with cell volume change and a decrease in intracellular K+[24]. In medulloblastoma cell line studies, forced expression of Kv10.2 in heterologous systems (HEK-293 cells) led to reduced cell volume and increased apoptosis [25]. In glioblastoma cells, KCa3.1 was involved when the cell death trigger activated the intrinsic (mitochondrial-mediated) pathway, and KCa1.1 was involved in activation of the extrinsic pathway that occurs in response to apoptotic triggers such as CD95 (also known as FAS) and Tumor Necrosis Factor (TNF) [24].

Contribution to tumor vascularization

Laboratory studies suggest that tumor cells that express Kv10.1 show significantly higher levels of Vascular Endothelial Growth Factor Receptor (VEGF) secretion than controls, and tumors that express Kv10.1 have increased vascularization [26]. In addition, Kv11.1 expression in glioblastoma increases VEGF secretion and appears to exert proangiogenic effects [27].

Potential use as prognostic markers

In a combined clinical and laboratory study of hematopoietic neoplasms Kv10.1 was also found to be up-regulated in myelodysplastic syndromes, Chronic Myeloid Leukemia (CML) and almost half of the tested Acute Myeloid Leukemia (AML) samples in a subtype-dependent fashion, suggesting that Kv10.1 may be a novel target for diagnostic, prognostic and/or therapeutic approaches in AML [28]. Other studies using clinical tumor samples suggest that Kv10.1 overexpression correlates with worse prognosis in head and neck cancer, ovarian cancer and soft tissue sarcoma [29-31].

Deranged epigenetic mechanisms

Aberrant methylation of K+ channel gene promoter regions is increasingly being recognized in a variety of cancer types [32]. Hypermethylation has been reported for: Kv10.2 in Non-Small Cell Lung Cancer (NSCLC) [33,34]; and Kv1.3 in breast cancer where it was associated with poorly differentiated tumors [35], and also in pancreatic adenocarcinoma [36]. In contrast, hypomethylation has been reported for Kca3.1 in NSCLC where it was also linked to worse prognosis [37].

Two-Pore Domain Potassium Channels and Cancer

K2p channels are made up of four transmembrane segments and 2 pores (P-loops) in tandem. The family of K2p channels has 15 members and these are thought to be background channels which enable the leak of K+ ions from cells and are open at rest [38]. Background or leak K+ conductance is known to stabilize the negative cellular resting membrane potential and counter balance inward depolarizing currents [39]. K2p channels are important for baseline cellular activity at rest including membrane potential, calcium homeostasis and cell volume regulation. Evidence largely from laboratory studies support the hypothesis that alterations of the expression or function of K2p channels in cancer cells may play a significant role in cancer development and progression [3,6,40].

The 15 K2p channel family members can be further subdivided into 6 structural and functional subfamilies [38]:

a. Two-pore, weakly inwardly rectifying (TWIK): TWIK-1 or KCNK1, TWIK-2 or KCNK6 and TWIK-3 or KCNK7

b. TWIK-related acid-sensitive: TASK-1 or KCNK3, TASK-3 or KCNK9 and TASK-5 or KCNK15

c. TWIK-related K+ channel: TREK-1 or KCNK2, TREK-2 or KCNK10; and TWIK-related, arachidonic acid-stimulated K+ channel: TRAAK or KCNK4

d. TWIK-related alkaline pH-activated K+ channels: TALK-1 or KCNK16 and TALK-2 or KCNK17; and TASK-2 or KCNK5

e. Tandem pore domain halothane-inhibited K+ channels: THIK-1 or KCNK13 and THIK-2 or KCNK12

f. TWIK-related spinal cord K+ channel TRESK or KCNK18

An online database search was performed to discover potential novel cancer relationships and tumorigenic mechanisms for all K2p genes and findings are provided below [41-43]. It should be noted that the following K2p genes have proximate chromosomal locations and potentially may exhibit marker correlation: KCNK1 (located on 1q42) and KCNK2 (1q41); KCNK3 (2p23) and KCNK12 (2p16); KCNK4 (11q13) and KCNK7 (11q13); KCNK5 (6p21), KCNK16 (6p21) and KCNK17 (6p21); and KCNK10 (14q31) and KCNK13 (14q32). In addition, a search of the NHGRI-EBI GWAS catalog was performed to describe any SNP-traits associated with cancer [44]. No SNP variants were found cataloged with associations with cancer for the following K2p genes: KCNK1, KCNK2, KCNK3, KCNK4, KCNK5, KCNK7, KCNK9, KCNK12, KCNK13, KCNK15, KCNK16, KCNK17 and KCNK18. SNP rs8102476 on KCNK6 was cataloged as associated with prostate cancer susceptibility [45]. SNP rs17124276 on KCNK10 was catalogued as associated with survival in pancreatic cancer [46]. A summary of K2p genes is provided in table 1.

Genomic Context[a]					Protein Context[a]	
Gene	**Alternate names**		**Location**	**Exons**	**AA**	**Function**
				#	**length**	
KCNK1	K2P1.1	TWIK1	1q42-q43	6	336	Probably nonfunctional; may require other proteins for activity
KCNK2	K2P2.1	TREK1	1q41	13	426	Functional; can be opened by certain anesthetics, membrane stretching, intracellular acidosis, and heat
KCNK3	K2P3.1	TASK1	2p23	3	394	Acid-sensitive potassium channel; activated by the anesthetics halothane and isoflurane
KCNK4	K2P4.1	TRAAK1	11q13.1	7	393	Channel is regulated by polyunsaturated fatty acids, temperature and mechanical deformation of the lipid membrane; protein is expressed primarily in neural tissues and may be involved in regulating the noxious input threshold in dorsal root ganglia neurons; naturally occurring read-through transcripts also exist between this gene and the downstream Testis Expressed 40 (TEX40) gene
KCNK5	K2P5.1	TASK2	6p21	5	499	Mainly expressed in the cortical distal tubules and collecting ducts of the kidney; protein is highly sensitive to external pH
KCNK6	K2P6.1	TWIK2	19q13.1	5	313	Widely expressed; stimulated by arachidonic acid, and inhibited by internal acidification and volatile anesthetics
KCNK7	K2P7.1	TWIK3	11q13	4	307	Probably nonfunctional; may require other proteins for activity
KCNK9	K2P9.1	TASK3	8q24.3	5	374	Amplification and overexpression of this gene have been observed in several types of human carcinomas; gene is imprinted in the brain, with preferential expression from the maternal allele; a mutation in this gene was associated with Birk-Barel mental retardation dysmorphism syndrome
KCNK10	K2P10.1	TREK2	14q31.3	10	538	Stimulated strongly by arachidonic acid and to a lesser degree by membrane stretching, intracellular acidification, and general anesthetics
KCNK12	K2P12.1	THIK2	2p16.3	3	430	Probably nonfunctional; may require other proteins for activity
KCNK13	K2P13.1	THIK1	14q32.11	2	408	Functions include regulating neurotransmitter release, heart rate, insulin secretion, neuronal excitability, epithelial electrolyte transport, smooth muscle contraction, and cell volume; open channel that can be stimulated by arachidonic acid and inhibited by the anesthetic halothane
KCNK15	K2P15.1	TASK5	20q13.12	2	330	Probably nonfunctional; may require other proteins for activity
KCNK16	K2P16.1	TALK1	6p21.2-p21.1	8	309	Gene is expressed predominantly in the pancreas and is activated at alkaline pH
KCNK17	K2P17.1	TALK2	6p21.1	7	332	Gene is activated at alkaline pH; KCNK3 is a paralog of this gene
KCNK18	K2P18.1	TRESK2	10q25.3	3	384	Mutation in this gene has been found to be associated with migraine with aura

Table 1: Two-pore domain potassium channel gene summary.

[a]From NCBI, GeneCards, PubMed and UCSC browser searches.

TWIK Subfamily: KCNK1, KCNK6, and KCNK7

The weakly inward rectifying channel KCNK1 or TWIK-1 was originally cloned from adult kidney and is expressed in many other tissues including brain and heart [47,48]. KCNK6 or TWIK-2 is the closest relative of KCNK1 and also has wide distribution [48,49]. Both are inhibited by pharmacological treatments known to lower intracellular pH [49]. KCNK7 or TWIK-3 however, has not been shown to be a functional channel and may require other non-pore-forming proteins for activity [38,50].

To date, none of these TWIK subfamily channels have been definitively implicated in having a role in cancer [3,38]. However, Williams et al., performed an exploratory online cancer microarray database study across several cancer types, comparing mRNA expression in cancer to normal tissue, and found the following results regarding the top 10% genes both over and under expressed [6]. When compared to normal tissue, significant overexpression of KCNK1 was observed in most cancers studied (including: bladder, brain, breast, cervix, esophageal, head and neck, kidney, leukemia, lung, lymphoma and pancreatic) while other cancer tissue types showed KCNK1 underexpression (including: melanoma, prostate and sarcoma). KCNK6 was overexpressed in breast and underexpressed in colorectal and esophageal cancers and melanoma.While KCNK7 failed to show overexpression in any of the cancer types examined, it showed significant underexpression in a range of cancers including: cervical, esophageal, head and neck, and lymphoma and melanoma [6].

TASK Subfamily: KCNK3, KCNK9 and KCNK15

The TASK (TWIK-related acid-sensitive) family of K+ channels share >50% of sequence identity, and within this family KCNK15 or TASK-5 has not been shown to be functionally expressed. Heterodimerization between KCNK3 or TASK-1 and KCNK9 or TASK-3 has been demonstrated, and TASK channels are expressed in the Central Nervous System (CNS) as well as heart (KCNK3, KCNK15) and adrenal gland (KCNK3, KCNK9) [49]. KCNK3 encodes a protein which is an outwardly rectifying channel that is sensitive to changes in extracellular pH and is inhibited by extracellular acidification. KCNK3 protein is found in brain and cerebellum and is activated by the anesthetics halothane and isoflurane, and blocked by bupivacaine [3]. Further, KCNK3 expression has been found to be down-regulated in patients with colorectal cancer with unfavorable prognosis, and as such KCNK3 may be considered a potential prognostic marker for colorectal cancer [51]. Like KCNK3, KCNK9 channels also generate outwardly rectifying currents that are modulated by a wide range of chemical and physical stimuli such as acidification and hypoxia, responds similarly to anesthetic agents and is normally observed in the brain with particularly strong expression in the cerebellum.

The KCNK9 gene is imprinted in brain tissue with preferential expression from the maternal allele, and a mutation in this gene is associated with Birk-Barel mental retardation dysmorphism syndrome [3,50,52]. Importantly, amplification of the KCNK9 gene has been reported in breast cancer (detailed further below) and melanoma [53,54]. KCNK9 has also been found to be overexpressed in breast, lung and colorectal cancer [54,55].

KCNK9 is recognized as a proto-oncogene and overexpression is known to promote tumor formation and induce resistance to hypoxia, and mice injected with NmuMG (mammary epithelial) cells overexpressing KCNK9 developed tumors [54]. Pei et al., established that the oncogenic potential of KCNK9 depends on K+ channel function and showed that a specific point mutation (G95E) abolished channel activity and abrogated its oncogenic functions, including proliferation in serum deprived media, resistance to apoptosis, and promotion of tumor growth. Hence wild-type KCNK9 confers a growth advantage to cells, whereas the inactivating mutant has no effect on cell growth [56]. Another study showed that KCNK9 channels were expressed in the mitochondria of melanoma cells, and were essential for maintaining cellular integrity and viability. They found that a KCNK9 knockdown melanoma cell line had altered morphology, reduced DNA content, decreased metabolic activity and impaired mitochondrial function [57].

Taken together, these reports suggest that KCNK9 channels play a key role in carcinogenesis [3,50]. Williams et al., found that KCNK3 showed altered expression in majority of cancers examined (13 out of 20) and was upregulated in leukemia, lymphoma, and kidney and breast cancer, and underexpressed in sarcoma and lung, pancreas, CNS, bladder, colorectal and prostate cancers.Whereas, KCNK9 only showed upregulated expression in breast cancer, and KCNK15 showed overexpression in breast and underexpression in gastrointestinal cancers.

TREK and TRAAK Subfamilies: KCNK2, KCNK10 and KCNK4

These channels are mechanically-gated and have been shown to open by membrane stretch. They are also opened by various lipids, including long chain polyunsaturated anionic fatty acids and neutral cone-shaped lysophospholipids [58]. KCNK2 or TREK-1 channels are activated by volatile anesthetics and may be an important target in the action of these drugs [59]. Increased KCNK2 expression has been found in prostate cancer samples compared to normal epithelium, and reduced proliferation of prostate cancer cell lines occurred when KCNK2 was experimentally knocked down [60].

A recent study evaluated KCNK2 expression in prostate cancer using Immunohistochemistry (IHC) and found that compared with normal prostate tissue, KCNK2 was overexpressed in cancer, and was also associated with less favorable clinical prognostic features [61]. Further, both KCNK2 and KCNK10 or TREK-2 are expressedin the normal human ovary and epithelial ovarian cancer, and modulators of KCNK2 in cell cultures have significant effects on cell proliferation and apoptosis [62]. KCNK10 is also overexpressed in bladder cancer cell lines and is thought to partly contribute to cell cycle-dependent growth [63].

A recent study demonstrates that KCNK4 or TRAAK is differentially methylated by race in breast cancer, with higher median levels being observed in non-Hispanic (nH) black, compared with nH white women (further elaborated on below) [64]. Williams et al., found that: KCNK2 was among the top genes overexpressed in lung and underexpressed in breast, gastrointestinal and head and neck cancers; KCNK10 was among the top genes underexpressed in colorectal, kidney, breast and brain cancers; and KCNK4 failed to show altered expression in any of the 20 cancers examined [6].

TALK and TASK-2 Subfamilies: KCNK16, KCNK17 and KCNK5

This subfamily comprises the TWIK-related alkaline pH-activated K+ channels plus the related TASK-2 channel, and expression of all 3 members (KCNK16 or TALK-1, KCNK17 or TALK-2 and KCNK5 or TASK-2) is very low in the CNS. These channels are stimulated by alkalinization of the external medium and are more present in peripheral tissues including kidney, liver, pancreas, and placenta for KCNK5, and mainly in the pancreas for KCNK16 and KCNK17. KCNK16 and KCNK17 are also strongly activated by Nitric Oxide Species (NOS) and Reactive Oxygen Species (ROS) [49]. In a systematic screening study to identify novel amplified cancer genes using 975 human cancer DNA samples, 750 cell lines and 225 primary tumors, KCNK5, KCNK17, and KCNK16 were among eight genes identified in a previously uncharacterized amplicon located on 6p21.2. However, these genes were not observed to be the most frequently amplified [65].

Another small study reported significant association between cumulative arsenic exposure and KCNK17 methylation levels in smoking-unrelated urothelial carcinoma [66]. Taken together, these findings which associate KCNK16 and KCNK17 with cancer are considered preliminary and need to be validated by further studies. KCNK5 expression has been examined in breast cancer and is discussed in detail below. Williams et al., found that: KCNK16 and KCNK17 failed to show convincing evidence of altered expression with the cancers examined; and KCNK5 was among the top upregulated genes in esophageal, breast and lung cancers, and among the top underexpressed genes in leukemia, sarcoma, and colorectal, kidney and liver cancers [6].

THIK Subfamily: KCNK13 and KCNK12

KCNK13 or THIK-1 and KCNK12 or THIK-2 are the tandem pore domain halothane-inhibited K+ channels and were originally isolated from rat brain. Despite a high level of sequence homology, KCNK13 produces background K+ currents, whereas KCNK12 appears to be silent. KCNK13 can be stimulated by arachidonic acid and inhibited by the anesthetic halothane [67]. A recent study however, shows that both KCNK13 and KCNK12 are active heteromeric channels, and that they can co-assemble and form functional channels in the plasma membrane [68]. To date, none of these THIK subfamily channels have been definitively implicated in having a role in cancer. Williams et al., found that there was evidence suggestive of KCNK13 overexpression in breast cancer, and KCNK12 overexpression in acute lymphocytic leukemia and lung cancer and underexpression in astrocytoma and glioblastoma [6].

TRESK Subfamily: KCNK18

KCNK18 or TRESK is the sole member of the TWIK-related spinal cord K+ channel subfamily and is regulated by the calcium/calmodulin-dependent protein phosphatase calcineurin and expression has been reported in the human spinal cord [69]. A study suggests that the background K+ currents in human lymphoma (Jurkat cells) are mediated by KCNK18 [70]. Further, a subsequent study showed that positive immunostaining for KCNK18 was demonstrated in lymphoblastic cell lines, in germinal centers of non-tumoral lymph nodes, and in clinical samples of T acute lymphoblastic leukemia/lymphoma, and the authors concluded that KCNK18 overexpression is related to immune system tumorigenesis [71]. Williams et al., found that when compared to normal tissue controls KCNK18 did not show altered expression in any of the cancer types examined [6].

The Role of K2p Channels in Breast Cancer

The role of K2p channel genes in breast cancer is currently emerging and several recent reviews suggest potential clinical utility of K2p channel genes as biomarkers in breast cancer [3,4,72]. Williams et al., examined the expression of all 15 K2p family members and showed that all but 5 showed altered expression in breast cancer (KCNK4, KCNK7, KCNK12, KCNK16, KCNK18). K2p genes KCNK1, KCNK3, KCNK5, KCNK6, KCNK9, KCNK13, KCNK15, and KCNK17 showed overexpression, while KCNK2 and KCNK10 showed underexpression [6].

KCNK4

A recent study examined DNA methylation at 1,287 CpG (cytosine-guanine) loci in the promoters of cancer-related genes in 216 nH black and 301 nH white women with invasive breast cancer. Results suggests that KCNK4 is differentially methylated according to black: white race, with higher median methylation beta values being observed for nH black women, whether using self-report or genotype markers for identification of race [64]. The authors also validated these findings using additional samples from The Cancer Genome Atlas (TCGA). It is however not clear from the report whether KCNK4 methylation is associated with a specific subtype, particularly as nH black patients in this study are known to have higher prevalence of aggressive basal (ER/PR: estrogen/progesterone receptor negative; HER2: human epidermal growth factor receptor-2 negative; and cytokeratin 5/6 positive and/or HER1 positive) type breast tumors.

KCNK5

In ER-alpha-positive cell lines (encoded by the ESR1), KCNK5 expression appears to be under the control of ER-alpha signaling. Specifically, researchers observed an up-regulation of transcript encoding KCNK5 in a screen for genes stimulated by 17 beta-estradiol (E2) in the ER-alpha-positive breast cancer cell lines MCF-7 and T47D. Further, chromatin immunoprecipitation assays revealed binding of ER-alpha to the KCNK5 enhancer region in E2-treated MCF-7 cells, and estrogen-responsive elements are present in the enhancer region of KCNK5. Cells treated with E2 also showed increases in the amplitude of pH-sensitive K+ currents which suggests that E2 treatment increases the number of active channels at the cell surface. Finally, the application of small interfering RNA specific for KCNK5 decreased pH-sensitive K+ currents and reduced the estrogen-induced proliferation of T47D cells, which confirms that this channel is required for normal E2-evoked proliferation of these cells [73]. In addition, Clarke et al., used a weighted gene co-expression network analysis in a recent study to identify co-regulated gene clusters across 2342 breast cancer samples from 13 microarray-based gene expression studies. In this study, 11 distinct co-expression clusters were identified from 5500 probe sets. In a cluster of genes that was found to correlate with prognosis exclusively for aggressive basal-like type breast cancer, upregulation of KCNK5 was associated with poor outcome for this subtype. The authors go on to suggest that KCNK5 may be a useful clinical marker for this subtype and that further research was needed [74].

KCNK9

Mu et al., first described the KCNK9 gene located at chromosomal region 8q24.3 and encoding TASK3 as a potential proto-oncogene. Amplification of the KCNK9 gene was detected in 10%, and overexpression of protein was detected in 44% of breast tumors, but not in normal tissue controls. Furthermore, overexpression of KCNK9 in cell lines promoted tumor formation and conferred resistance to hypoxia, suggesting that amplification and overexpression of KCNK9 provides selective advantage to breast cancer cells [54]. In addition, a subsequent study established a direct link between K+ channel activity of KCNK9 and its proliferative oncogenic function; specifically, K+ channel activity was required for KCNK9 to promote tumor formation in nude mice, and a dominant-negative mutant for expression of the mutant protein inhibited tumorigenicity of wild-type KCNK9 [56]. As such, KCNK9 has been found to be frequently amplified in breast tumors where it increases proliferation and migration in a K+ permeation-dependent manner [4,75].

KCNK9 is a maternally imprinted gene with monoallelic expression predominantly in brain tissue, but expression has been observed in breast tissue - both are of ectodermal origin [52]. Hence it is theorized that as KCNK9 is a maternally imprinted gene, overexpression may occur due to relative Loss of Methylation (LOM) and subsequent functionally biallelic expression which may be equivalent to duplication of an active allele [76]. Early studies have reported frequent LOM at KCNK9 differentially methylated regions with biallelic expression in some breast tumor biopsies and random periareolar fine-needle aspirates from non-cancerous breast in high-risk, nH black women, and also that LOM is associated with more aggressive triple-negative (TN; negative for ER/PR/HER2 status determined by IHC) breast cancer [76]. Furthermore, it has been suggested that for TN disease, nH black women have higher KCNK9 LOM levels compared to nH white, 90% versus 67% respectively [76]. However, the epidemiologic data to date characterizing KCNK9 is limited [52,76-78].

KCNK12

Molecular changes involved in histologic tubular breast carcinoma were examined using microarray-based comparative genomic hybridization focusing on 287 genomic target clones of oncogenes and tumor suppressor genes in 21 patient samples of tubular carcinoma. The highest frequencies for DNA sequence copy number losses were detected for CDH13 and MSH2/KCNK12 (in 86% and 52% of the samples, respectively). However, because it was not the most prominent copy number change location, KCNK12 loss was not further evaluated in the study [79].

Analysis of The Cancer Genome Atlas (TCGA) data

Recently Dookeran et al., systematically evaluated associations of K2p gene expression and DNA methylation with TN subtype using TCGA invasive breast cancer data. Overexpression of KCNK5, KCNK9 and KCNK12, and underexpression of KCNK6 and KCNK15, were significantly associated with TN vs. luminal A (ER/PR+ and HER2-) subtype (Bonferroni-corrected $p < 0.0033$). A total of 195 (114 hypomethylated and 81 hypermethylated) CpG loci were found to be significantly associated with TN subtype (Bonferroni-corrected $p < 8.22 \times 10^{-8}$). Significantly negatively correlated expression patterns that were differentially observed in TN vs. luminal A subtype were demonstrated for KCNK2, KCNK5 and KCNK9. CpG loci listed for KCNK5 and KCNK9 all showed relative hypomethylation for probability of TN vs. luminal A subtype. Regarding specificity for association of K2p expression with tumor vs. normal sample-type, the MEXPRESS web tool was used to visualize expression and clinical TCGA data [80], and showed that KCNK9 and KCNK12 overexpression appeared to be associated with tumor type, while KCNK5 overexpression appeared to have marginal association. Sensitivity analyses that examined associations of K2p expression and other subtypes related to TN status (ER/PR-negative, basal and Integrative Cluster type 10) also showed consistency with similar mRNA expression patterns. The authors concluded that TN subtype was associated with distinct K2p expression patterns, and both KCNK5 and KCNK9 overexpression appeared to be functionally related to CpG loci hypomethylation [81]. However, additional examination of breast cancer recurrence/progression for select TCGA

K2p gene expression did not reveal significant associations in age and race adjusted Cox models (Table 2).

K2p Gene	Adjusted[a]				
	Tertile[b]	HR	95% CI		p-value
KCNK5	1 (ref)	…	…	…	…
	2	0.70	0.39	1.28	0.249
	3	0.79	0.45	1.40	0.425
KCNK6	1 (ref)	…	…	…	…
	2	1.45	0.81	2.59	0.212
	3	1.21	0.68	2.17	0.515
KCNK9	1 (ref)	…	…	…	…
	2	1.11	0.60	2.03	0.742
	3	1.22	0.69	2.17	0.493
KCNK12	1 (ref)	…	…	…	…
	2	1.45	0.80	2.65	0.223
	3	1.39	0.75	2.60	0.297
KCNK15	1 (ref)	…	…	…	…
	2	1.07	0.61	1.85	0.823
	3	0.89	0.49	1.61	0.698

Table 2: Cox models for breast cancer recurrence/progression for select TCGA K2p gene expression.

[a]Cox models adjusted for continuous age and nH black vs. white race.

[b]Gene expression modeled as tertiles to examine linearity of response.

K2p, two pore domain potassium channels; HR, hazard ratio; nH, non-Hispanic.

Summary and Future Directions

Taken together, these reports suggest that the K2p family of K+ channel genes are potential novel molecular markers in breast cancer. Several associations have been suggested in clinical breast cancer: specifically, aberrant DNA methylation status (KCNK4, KCNK5 and KCNK9), copy number change (KCNK12 and KCNK9) and altered mRNA expression (particularly overexpression of KCNK5, KCNK9 and KCNK12). Associations have also been demonstrated with functional hormone-receptor status (KCNK5), TN and basal subtype (KCNK5, KCNK9 and KCNK12), and nH black race (KCNK9), which may be indicative of involvement in subtype related mechanistic pathways. Further systematic evaluation of K2p genes may be worthwhile as these features may be related to biologically aggressive breast tumor type. Prior experience gained with pharmacological manipulation of K+ channels in other pathologies might facilitate the use of K+ channels as oncologic molecular-targets in precision-medicine [3,4]. Wallace et al., suggest that K+ channel activity may represent a mechanism through which phytoestrogens act (e.g., Genistein) [82].

Another study in TN breast cancer cells suggests that inhibition of intermediate-conductance KCa channel KCNN3 with specific blockers including the antifungal clotrimazole, suppressed cell proliferation, migration and epithelial-mesenchymal transition [83]. Sun et al., suggest that antibody-based KCNK9 targeting is a promising therapeutic strategy in KCNK9 overexpressing malignancies and showed that a monoclonal antibody (Y4) against KCNK9 extracellular-domain effectively inhibits growth of human lung cancer xenografts and breast cancer metastasis in mice [84]. Investigators have also been researching whether loss of KCNK9 imprint-control and/or differential methylation could be adapted in novel approaches for clinical diagnosis and targeted therapy in aggressive TN breast cancer [52,76-78]. Current molecular epidemiologic data characterizing K2p channel genes in breast cancer is however limited, and lacks a systematic examination approach, with few peer-review publications, and this is considered a critical knowledge deficiency to progress in the field.

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