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TOPICAL REVIEWS

The Life and Death of Breast Cancer Cells: Proposing a Role for the Effects of Phytoestrogens on Potassium Channels

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Abstract Changes in the regulation of potassium channels are increasingly implicated in the altered activity of breast cancer cells. Increased or reduced expression of a number of K^+ channels have been identified in numerous breast cancer cell lines and cancerous tissue biopsy samples, compared to normal tissue, and are associated with tumor formation and spread, enhanced levels of proliferation, and resistance to apoptotic stimuli. Through knockout or silencing of K^+ channel genes, and use of specific or more broad pharmacologic K^+ channel blockers, the growth of numerous cell lines, including breast cancer cells, has been modified. In this manner it has been proposed that in MCF7 breast cancer cells proliferation appears to be regulated by the activity of a number of K^+ channels, including the Ca^{2+} activated K^+ channels, and the voltage-gated K^+ channels hEAG and $K_v1.1$. The effect of phytoestrogens on K^+ channels has not been extensively studied but yields some interesting results. In a number of cell lines the phytoestrogen genistein inhibits K^+ current through several channels including $K_v1.3$ and hERG. Where it has been used, structurally similar daidzein has little or no effect on K^+ channel activity. Since many K^+ channels have roles in proliferation and apoptosis in breast cancer cells, the impact of K^+ channel regulation by phytoestrogens is of potentially great relevance.

Keywords Breast cancer · Daidzein · Genistein · hEAG · hERG · Isoflavone · $K_v1.1$ · $K_v1.3$ · MCF7 · Potassium channel

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The membrane potential of a cell is the difference in voltage between the interior and exterior of the plasma membrane. Because the bilipid membrane itself is essentially impermeable to ions, the membrane potential arises from the actions of various ion channels and pumps embedded in the lipid bilayer. Most these channels regulate the flow of K^+, Na^+, Cl^- and Ca^{2+} ions. Ion channels are classified according to how they are regulated. The main groups consist of ligand gated channels, voltage gated channels, channels which respond to sensory stimuli such as stretching or temperature, and finally leakage, or rectifier channels. The latter group are the simplest, with very little in the way of regulation, although they frequently operate better in one direction than the other, and may be closed by some ligands. In addition to being gated in these manners, most voltage and ligand gated channels are susceptible to regulation by tyrosine phosphorylation. This allows intracellular signaling pathways and growth factors to acutely regulate the electrophysiologic properties of both excitable and nonexcitable cells. Further information regarding the activities of ion channels can be found in the comprehensive handbook by Hille (2001).

In excitable cells such as neurons or muscle cells, ion channels are used to generate action potentials, where an electric current transmits signals through the cell. However, in nonexcitable cells, including breast cancer cells, regulation of membrane potential is key to many other processes including determination of membrane potential and the rate of repolarization, osmolarity, proliferation and apoptosis (Davis et al. 2001; Felipe et al. 2006; Ouadid-Ahidouch and Ahidouch 2008).

Potassium channels are the largest and most diverse family of ion channels. They show cell and tissue specific regulation of expression levels, and play well established roles in many diseases such as congenital deafness,

arrhythmias, and multiple sclerosis (Felipe et al. 2006). In addition, the deregulation of potassium channels has been implicated in the development and progression of breast cancer (Ouadid-Ahidouch and Ahidouch 2008; Wang 2004; Wonderlin and Strobl 1996). Table 1 lists all the K^+ channels referred to within this text, together with their International Union of Pharmacology (IUPHAR) assigned names (International Union of Pharmacology 2011) and gene names established by the HUGO Gene Nomenclature Committee (2011). For an exhaustive list of all known K^+ channels consult the IUPHAR database.

Potassium channels can be broadly grouped into four families (Felipe et al. 2006). These are the voltage gated, calcium-activated, inward rectifier (K_{ir}), and two pore domain K^+ channels (K_{2P}). The voltage gated potassium channels (VGKCs) form the largest group, comprising the peptides K_v1 to K_v12 (Gutman et al. 2005). Each K_v peptide forms a channel subunit, of which four are required to act as a functional K^+ channel. These can be as homo- or heterotetramers. The activity of these channels is voltage dependent. They tend to be closed at resting potential and open upon membrane depolarization to mediate an outward K^+ current, resulting in hyperpolarization.

There are two groups of calcium-activated K^+ (K_{Ca}) channels. The small conductance (SK) and intermediate conductance (IK) calcium activated channels are voltage insensitive and are activated by low concentrations of internal calcium (<1 μM). These channels do not directly bind Ca^{2+}, but instead detect it using a calmodulin dependent mechanism. On the contrary, the large conductance (BK) calcium activated K^+ channel is activated by voltage and internal Ca^{2+}. The latter is detected not via calmodulin, but probably through interaction with several cation binding sites on the C terminal domain of each channel subunit (Wei et al. 2005).

The K_{2P} group can be regulated by a range of chemical and physical stimuli including pH, mechanical stretch, lipids, and various ligands. They are active at resting potentials, and mediate background, or "leak" outwardly, rectifying K^+ currents that stabilize membrane potential and allow repolarization (Enyedi and Czirjak 2010; Goldstein et al. 2005). Finally, the K_{ir} channels mediate an inward K^+ current activated upon hyperpolarization (Kubo et al. 2005).

In MCF7 breast cancer cells many plasma membrane K^+ channels have been identified, and are summarized in Table 2. A number of these channels have roles in the proliferation of breast cancer cells, and their overexpression is associated with the promotion of tumor formation and resistance to apoptotic stimuli (Abdul et al. 2003; Brevet et al. 2008; Mu et al. 2003).

Of particular note is the hEAG (human *ether-à-go-go*) K^+ channel, also known as KCNH1 or $K_v10.1$. This was found, by use of both reverse transcription real-time PCR and immunostaining, to be overexpressed in over 80% of breast carcinoma biopsy samples. In normal tissue its distribution was restricted to areas of the brain and several other tissues (Hemmerlein et al. 2006).

A related channel is hERG (human *ether-à-go-go*-related gene), also known as KCNH2 or $K_v11.1$. This channel carries a rapidly activated delayed rectifier K^+ current. Like hEAG, hERG is expressed in numerous human cancer cell lines and tissues but not in corresponding healthy cells (Bianchi et al. 1998; Cherubini et al. 2000; Lastraioli et al. 2004; Pillozzi et al. 2002), suggesting that it may also

Table 1 Potassium channel nomenclature and genes

IUPHAR[a]	HUGO[b]	Other names
$K_v1.1$	*KCNA1*	Shaker-related
$K_v1.2$	*KCNA2*	
$K_v1.3$	*KCNA3*	
$K_v1.5$	*KCNA5*	
$K_v1.6$	*KCNA6*	
$K_v10.1$	*KCNH1*	Human *ether-à-go-go* K^+ channel, hEAG, eag1
$K_v11.1$	*KCNH2*	Human *ether-à-go-go*-related gene, hERG, erg1
$K_{Ca}1.1$	*KCNMA1*	Large conductance calcium-activated K^+ channel, BK, BK_{Ca}
$K_{Ca}2.1$	*KCNN1*	Small conductance calcium-activated K^+ channel, SK, SK_{Ca}
$K_{Ca}2.2$	*KCNN2*	
$K_{Ca}2.3$	*KCNN3*	
$K_{Ca}3.1$	*KCNN4*	Intermediate conductance calcium-activated K^+ channel, IK, IK_{Ca}
$K_{2P}9.1$	*KCNK9*	Two-pore domain K^+ channel subunit, TWIK-related acid sensitive channel 3, TASK3
$K_{ir}3.1$	*KCNJ1*	G-protein inwardly rectifying K^+ channel, GIRK1
$K_{ir}3.2$	*KCNJ2*	G-protein inwardly rectifying K^+ channel, GIRK2
$K_{ir}3.3$	*KCNJ3*	G-protein inwardly rectifying K^+ channel, GIRK3

[a] Protein names assigned by the International Union of Pharmacology (2011)

[b] HUGO Gene Nomenclature Committee (2011) approved gene name

Table 2 Potassium channels found in MCF7 cells

Channel family	K^+ channel	References
Voltage gated	hEAG Human *ether-à-go-go* $K_v10.1$	Borowiec et al. 2007; Hemmerlein et al. 2006; Roy et al. 2008
	hERG Human *ether-à-go-go*-related gene $K_v11.1$	Bianchi et al. 1998; Chen et al. 2005; Roy et al. 2008; Wang et al. 2002
	$K_v1.1$	Ouadid-Ahidouch et al. 2000
Ca^{2+} activated	IK Intermediate Ca^{2+} activated channel	Ouadid-Ahidouch et al. 2004b
	BK Large Ca^{2+} activated channel	Khaitan et al. 2009; Ouadid-Ahidouch et al. 2004a; Roger et al. 2004
	SK Small conductance Ca^{2+} activated K^+ channel	Abdul et al. 2003
Inwardly rectifying	$K_{ir}3.1$ GIRK1 (G-protein inwardly rectifying)	Dhar and Plummer 2006
	$K_{ir}3.2$ GIRK2	
	$K_{ir}3.3$ GIRK3	

confer some selective advantage to the tumor cells. In cancer cell lines hERG expression varies greatly, but appears to relate to chemosensitivity, with the highest expression levels corresponding with the greatest sensitivity to anticancer drugs (Chen et al. 2005). In colonic cancers hERG expression level and activity appears to correlate with the invasiveness of the cancer (Lastraioli et al. 2004).

Similarly, the Shaker family potassium channel subunit $K_v1.3$ was detected by immunostaining in high or moderate levels in nearly 90% (53 out of 60) of breast cancer biopsy epithelial samples, while none was detected in four corresponding noncancerous samples (Abdul et al. 2003). However, Brevet et al. (2008) found the opposite, suggesting that both $K_v1.3$ and related $K_v1.1$ proteins were present at lower levels in cancerous tissue than normal tissue. For their study they immunostained 33 primary invasive breast carcinomas of varying stages and invasiveness, and 31 normal breast specimens. They related the reduction in $K_v1.1$ and $K_v1.3$ to their role in apoptosis in breast epithelial cells. $K_v1.3$ channel protein was not detected in MCF7 cells by immunohistochemical analysis (Ouadid-Ahidouch et al. 2000).

Jang et al. (2009) proposed that this discrepancy in results may be because expression of the $K_v1.3$ gene depends upon the tumorigenicity and stage of the cancer. This group found that weakly tumorigenic M13SV1R2 cells showed considerable and significantly greater $K_v1.3$ mRNA expression levels than in normal, untransformed M13SV1 cells, but that in the highly tumorigenic line M13SV1R2-N1, $K_v1.3$ gene expression was half of that seen in the normal line. They also discovered that compared to normal breast tissue, the expression of $K_v1.3$ was only higher during early (I, IIA, and IIB), late (IIIC), and metastatic (IV) stage breast cancer tissue. Expression during the mid-stages IIIA and IIIB, was not significantly different from normal tissue. However, Jang et al. (2009) also analyzed protein levels of $K_v1.3$ using Western blot analysis, and found that this was significantly higher in both the weakly and highly tumorigenic cell lines compared to the untransformed cells, and that protein levels were related to the tumorigenicity of the cell line. $K_v1.3$ protein levels did not correlate with mRNA levels. This is not an uncommon occurrence, and suggests differences in translation regulation or protein half-life.

This data suggests a possible explanation for the discrepancies in results between Abdul et al. (2003) and Brevet et al. (2008). However, it must be noted that the latter group did not find any significant relationships between the levels of $K_v1.1$ or $K_v1.3$ and markers of tumor grade or invasiveness, such as estrogen receptor (ER) status or Ki67 levels. Clearly further research into the regulation of $K_v1.3$ is required. Whether the regulation of $K_v1.3$ levels according to tumor stage and tumorigenicity can account for the apparent absence of $K_v1.3$ in MCF7 cells reported by Ouadid-Ahidouch et al. (2000) is not known.

Similarly, the K^+ channel $K_{2P}9.1$, encoded by the gene *KCNK9,* was found to be overexpressed at least 5-fold and up to over 100-fold in 44% (28 out of 64) breast cancer biopsy samples (Mu et al. 2003). It was also overexpressed in 35% of lung cancer samples analyzed (Mu et al. 2003). In the breast cancer samples, the *KCNK9* locus was amplified between 3- and 10-fold in 10% of the samples. Immunohistochemical analysis of the same samples confirmed the presence of high levels of $K_{2P}9.1$ protein in the samples where the gene was overexpressed, and Mu et al. (2003) also found overexpression of *KCNK9* to be associated with tumor formation, increased viability in low-serum conditions, and resistance to hypoxia.

$K_{ir}3.1$ has been found to be overexpressed in cancerous breast tissue compared to normal tissue, using immunostaining methods (Brevet et al. 2008). Unlike the other K^+ channels differentially expressed in cancerous compared to normal breast tissue, which mediate outward K^+ currents, $K_{ir}3.1$ facilitates an inwardly rectifying K^+ current. $K_{ir}3.1$ levels relate to tumor grade, with significantly higher expression seen in grade II than grade III tumors (Brevet et al. 2008). These results agree with those of Stringer et al. (2001), who used a gene expression profiling technique with a paired sample of breast carcinoma and adjacent normal breast tissue from the same patient, followed by RT-PCR with 56 separate benign and invasive breast carcinomas and 6 normal, nonmalignant breast tissue samples. In the latter investigation, $K_{ir}3.1$ overexpression was found to correlate significantly with the presence of lymph node metastasis. $K_{ir}3.1$ has also been identified by both immunostaining and real time RT-PCR in a number of breast cancer cell lines including MCF7, MDA-MB-453, and ZR-75–1 (Dhar and Plummer 2006).

Of the SK channels, the expression of SK1 (*KCNN1*) is restricted to neuronal tissues (Chen et al. 2004). Although SK2 (*KCNN2*) is more widespread, there remains very little or no expression in the mammary epithelium. On the contrary, SK3 (*KCNN3*) was detected in almost every tissue tested, including the mammary gland. However this study was limited to healthy, not cancerous tissue samples. To the knowledge of the authors, protein or expression levels of the SK channels have not yet been compared between healthy and cancerous breast epithelial cells.

Similarly, the IK channel (*KCNN4*) mRNA is expressed in the normal mammary gland (Chen et al. 2004), but no comparison to cancerous breast tissue has been made by this group. IK channel mRNA, protein, and functional channel activity have been detected in human breast cancer epithelial primary cell cultures and breast cancer tissue samples (Haren et al. 2010). However, no noncancerous controls were used for comparison so how the levels of IK compare between cancerous and noncancerous breast tissue is not known. However, Haren et al. (2010) also demonstrated that IK expression level correlates significantly with tumor grade, indicating that this channel may contribute to tumor formation or progression.

Khaitan et al. (2009) found very low levels of both expression and protein levels for the BK channel in normal mammary tissue and untransformed mammary cell line MCF10A. They found slightly higher expression levels primary breast cancer tissue samples ($n = 6$). On the contrary, Brevet et al. (2008) found lower levels of BK protein among 33 primary invasive ductal breast carcinomas compared to normal breast tissue from the same specimens. Regrettably, the low numbers of specimens used by both groups makes it impossible to reach a definitive conclusion regarding the relative levels of BK in cancerous and normal breast tissue at this stage. However, BK expression appears to be considerably higher among tissue samples of breast cancer metastasised to other organs, particularly the brain ($n = 4$), suggesting a role for the BK channel in brain metastasis (Khaitan et al. 2009).

Role of Potassium Channels in the Proliferation of MCF7 Breast Cancer Cells

K^+ currents are frequently studied in the ER-positive MCF7 breast cancer cell model. Treatment of MCF7 cells with the K^+ channel opener minodoxil resulted in an increase in cell proliferation (Abdul et al. 2003). In addition, treatment of MCF7 cells with a number of specific and nonspecific K^+ channel blockers results in inhibition of proliferation, as summarized in Table 3. This data implicates involvement of the SK channels, ATP sensitive channels and the voltage-gated channels (Pardo 2004; Wonderlin and Strobl 1996) in the proliferation of MCF7 cells. Which particular ATP-sensitive K^+ channels this relates to does not appear to be known.

Incubation with iberiotoxin or charybdotoxin (both Ca^{2+} activated K^+ channel blockers) had no effect on MCF7 proliferation, even at doses far in excess of their IC_{50} (dose required for inhibition of the maximal response by 50%) for the reduction of K^+ channel activity (Abdul et al. 2003; Ouadid-Ahidouch et al. 2000, 2004a). Neither did E-4031, a specific hERG blocker, have any effect (Roy et al. 2008). Given that astemizole treatment reduces MCF7 proliferation and is known to block both hERG and hEAG, this connects hEAG, but not hERG, with the proliferation of these cells.

In addition to hEAG, another VGKC potentially involved in the proliferation and cell cycle progression of MCF7 cells is $K_v1.1$. This channel is known to be expressed in these cells, while a number of other VGKCs, including $K_v1.2$ and $K_v1.3$, have not yet been identified, or are absent (Ouadid-Ahidouch et al. 2000; Ouadid-Ahidouch and Ahidouch 2008).

Table 3 Effects of K^+ channel blockers on the proliferation and K^+ channel activity of MCF7 breast cancer cells

Channel blocker	Channels affected	Inhibition of MCF7 proliferation	K^+ channel activity IC_{50}	References
4-Amino-pyridine (4AP)	VGKCs	IC_{50} 1.6 mm[a]	Between 0.1 and 4 mm[b]	Grissmer et al. 1994; Teisseyre and Michalak 2005; Wonderlin and Strobl 1996; Yao and Kwan 1999
α-Dendrotoxin (α-DTx)	$K_v1.1$	30% inhibition: 10 nM	0.6 nM	Ouadid-Ahidouch et al. 2000
Amiodarone	Nonspecific ion channel blocker	IC_{50} 1 μM (approximately)		Abdul et al. 2003
Astemizole (Ast)	hEAG hERG	IC_{50}30 μM	hEAG: 200 nM[b] hERG: 1.5 nM[b]	Borowiec et al. 2007; Garcia-Ferreiro et al. 2004; Ouadid-Ahidouch et al. 2004b; Roy et al. 2008; Salata et al. 1995
Charybdotoxin (CTx)	BK and IK	No effect up to 100 nM	50 nM	Coiret et al. 2005; Ouadid-Ahidouch et al. 2000; Ouadid-Ahidouch et al. 2004a, b
Clotrimazole	IK	25% inhibition: 5 μM	2 μM (approximately)	Ouadid-Ahidouch et al. 2004b
Dequalinium	SK	IC_{50} 1 μM (approximately)		Abdul et al. 2003
E-4031	hERG	No effect up to 300 nM	7.7 nM[b]	Roy et al. 2008; Zhou et al. 1998
Glibenclamide	ATP-sensitive K^+ channels	IC_{50} 50 μM	Pancreatic K_{ATP} channels; 0.48 nM[b] Cardiac K_{ATP} channels; 51 nM[b]	Abdul et al. 2003; Stephan et al. 2006; Wonderlin and Strobl 1996
Iberiotoxin (IbTx)	BK	No effect up to 500 nM	100 nM (approximately)	Coiret et al. 2005; Ouadid-Ahidouch et al. 2000; Ouadid-Ahidouch et al. 2004a, b
Tetraethyl-ammonium (TEA)	BK Voltage-activated K^+ channels ATP-sensitive K^+ channels	IC_{50} 5.8 mM	5 mM (500 μM blocks BK channel)	Coiret et al. 2005, 2007; Grissmer et al. 1994; Ouadid-Ahidouch et al. 2004a, b; Wonderlin and Strobl 1996; Yao and Kwan 1999

[a] Doses ≤ 1 mM were not found to inhibit MCF7 proliferation (Abdul et al. 2003)

[b] IC_{50} not determined in MCF7 cells

Care must be taken when extrapolating the IC_{50} data summarized in Table 3, as in several cases (denoted by [b]) no data exists for MCF7 cells. However, in general, the data for MCF7 cells corresponds with other cell lines (Wonderlin and Strobl 1996). This highlights areas where the effects of these compounds in MCF7 require further characterization.

Where dose-response relationships have been studied, the IC_{50} for inhibition of proliferation is frequently higher than the IC_{50} for inhibition of K^+ current activity. This suggests that inhibition of proliferation may be through nonspecific, or cytotoxic actions of the channel blocker, rather than through inhibition of K^+ channel activity, or that the effects on channel activity contribute a proportion of the response only. However, serum is added to the proliferation culture media but not the solutions used to record K^+ movement, and it is thought that components of the serum (e.g. albumin) may either bind the channel blocker, reducing its effectiveness, or itself further promote proliferation (Wonderlin and Strobl 1996). In addition, where these channels have been ablated by silencing the gene (Koeberle et al. 2010; Weber et al. 2006), or transfected into cells known to not normally express them, similar results have been achieved (Cayabyab and Schlichter 2002; Dong et al. 2010; Gierten et al. 2008; Grissmer et al. 1994; Szabo et al. 2008; Zhang and Wang 2000). These studies add strength to the argument

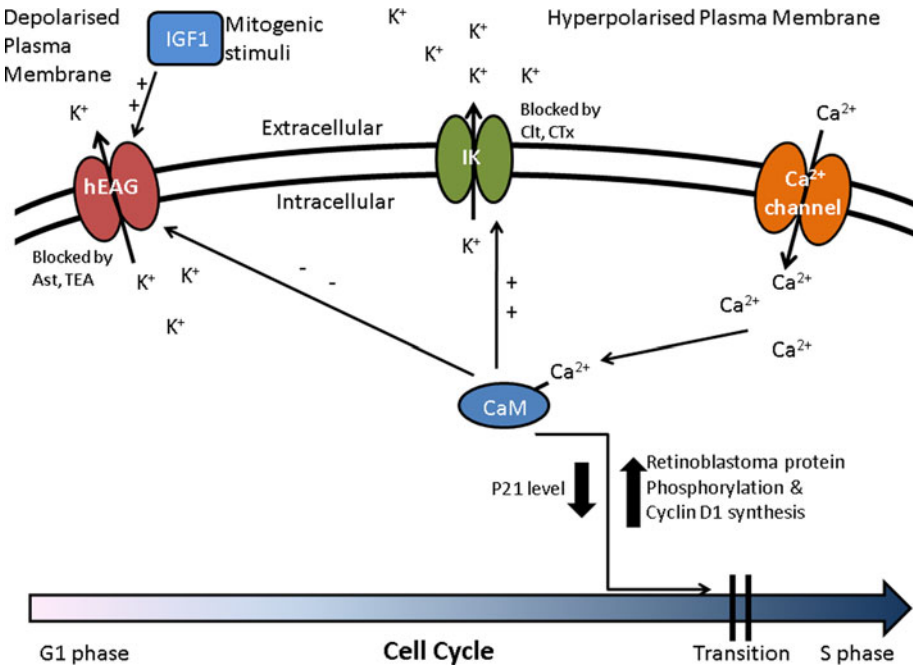

Fig. 1 Membrane potential model of breast cancer cell proliferation. *Ast* astemizole, *CaM* calmodulin, *Clt* clotrimazole, *CTx* charybdotoxin, *hEAG* human *ether-à-go-go* K^+ channel, *IGF1* insulin-like growth factor 1, *IK* intermediate conductance K^+ channel, *TEA* tetraethyl ammonium

that the channel blockers inhibit proliferation through blockade of K^+ channels, rather than through nonspecific mechanisms.

Work is ongoing to understand the mechanisms through which these channels and channel blockers affect proliferation in breast cancer cells (Fig. 1). Insulin-like growth factors such as IGF1 are important regulators of mammary gland proliferation at key developmental stages including pregnancy, lactation and involution (Hadsell 2003), and play vital roles in the initiation and progression of breast cancer (Jin and Esteva 2008; Weinstein et al. 2009). Treatment of MCF7 with the growth factor IGF1 results in an increase in proliferation associated with a rapid increase in K^+ current, membrane hyperpolarization, and an increase in levels of the K^+ channel hEAG mRNA (Borowiec et al. 2007). This IGF1-stimulated increase in proliferation was prevented by astemizole treatment, showing that hEAG is not only regulated by IGF1, but also plays a vital role in IGF1 mitogenic signalling in breast cancer cells. However, as discussed, astemizole is a relatively nonspecific channel blocker, and has been shown to block hERG also (Pardo et al. 2005; Roy et al. 2008). It is possible that the results of these studies may reflect the combined activities of the two channels, although the lack of any effect of the hERG antagonist E4031 may suggest that hEAG is physiologically more important in this instance.

Activation of hEAG in MCF7 cells, by membrane depolarization to potentials higher than −20 mV, results in membrane hyperpolarization. This is associated with cell cycle progression from G1 to S phase (Borowiec et al. 2007; Ouadid-Ahidouch and Ahidouch 2008; Ouadid-Ahidouch et al. 2004b; Strobl et al. 1995). Membrane hyperpolarization is generally accepted to be involved in cell cycle progression, in what is known as the "membrane potential model" of proliferation (Ouadid-Ahidouch and Ahidouch 2008; Pardo 2004). Blocking hEAG or IK by silencing or use of inhibitors astemizole (blocks hEAG and hERG) and clotrimazole (IK blocker), leads to membrane depolarization, reduced intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and accumulation of the cell cycle progression inhibitor p21 (Ouadid-Ahidouch and Ahidouch 2008; Ouadid-Ahidouch et al. 2004b). Their inhibitory effect was additive, but blocking hEAG resulted in greater proliferation inhibition and G1 phase arrest, than did blocking IK, leading to the suggestion that progression through G1 toward S phase is dependent on hEAG activity, while IK regulates membrane potential at the G1/S transition.

The relationship between calcium and potassium channels in breast cancer cells is complex, at times paradoxical, and poorly understood. Both hEAG and IK are regulated by an increase in $[Ca^{2+}]_i$ and calmodulin (CaM), a Ca^{2+} binding protein. However, while hEAG activity is inhibited by Ca^{2+}/CaM binding to its N-terminal domain (Ziechner et al. 2006), IK is activated by Ca^{2+}/CaM (Fanger et al. 1999). These effects can occur simultaneously in MCF7 cells (Ouadid-Ahidouch and Ahidouch 2008).

Calmodulin is required for the proliferation of numerous breast cancer cell lines including MCF7, T47D and MDA MB 231, regardless of 17β-estradiol (E2) treatment or ER status. This is demonstrated by incubation with CaM

antagonists such as ethoxiyl-butyl-berbamine (Shi et al. 2011). In addition, inhibition of calcium-calmodulin-dependent kinases (CaM-Ks) by small interfering RNA (siRNA) or antagonists also reduced proliferation and caused G1 phase arrest in MCF7 cells (Rodriguez-Mora et al. 2005), possibly by inhibiting cyclin D1 synthesis and retinoblastoma protein phosphorylation. Interestingly, CaM also binds to the ERs, increasing their stability and cellular levels, in a Ca^{2+}-dependent, E2-independent manner (Li et al. 2001), and calcium-calmodulin-dependent kinase IV was determined to be activated by ERα/E2 interaction in MCF7 cells, although not by ERα in combination with resveratrol or a number of xenoestrogens (Li et al. 2006).

It is likely that there are other factors involved in the regulation of hEAG and IK besides Ca^{2+}/CaM. However, Ouadid-Ahidouch and Ahidouch (2008) propose the following basic model for their role in proliferation, which may be summarized as follows: in early G1 phase, membrane potential is depolarized (around −20 mV) and $[Ca^{2+}]_i$ is low, resulting in the activity of hEAG but not IK. Mitogenic stimuli results in an increase in hEAG expression and therefore activity. The result is membrane hyperpolarization as G1 phase progresses. This causes an influx of Ca^{2+} into the cell, simply by controlling the electrochemical gradient. Increasing levels of $[Ca^{2+}]_i$ and Ca^{2+}/CaM activate IK resulting in stronger membrane hyperpolarization. As G1 progresses to S phase, CaM and the CaM-Ks are also involved in the regulation of levels of cell cycle proteins such as cyclin D1 and p21. The end result is progression to S phase, and enhanced proliferation of breast cancer cells.

In MCF7 cells, a noninactivating outward K^+ current was found which was inhibited dose- and voltage-dependently by α-dendrotoxin (α-DTx; a toxin from the black mamba snake *Dendroaspis augusticeps*), with maximal inhibition being obtained at 10 nM α-DTx after approximately 7 min of treatment, and an IC_{50} of 0.6 ± 0.3 nM (Ouadid-Ahidouch et al. 2000). Alpha-DTx blocks the channels $K_v1.1$, $K_v1.2$ and $K_v1.6$ (Harvey and Robertson 2004). RT-PCR and immunocytochemical methods have shown that $K_v1.1$ is present in MCF7 cells, but anti-$K_v1.2$ antibodies did not label these cells (Ouadid-Ahidouch et al. 2000), indicating that $K_v1.1$ may be the pharmacologic target of α-DTx in this case. $K_v1.6$ was not included in this examination, and it is not known whether this channel is present in MCF7. [^{3}H]-Thymidine DNA incorporation was used to determine that α-DTx inhibited MCF7 proliferation in a dose-dependent manner, at the same doses which inhibited K^+ current. Ouadid-Ahidouch et al. (2000) suggest that this implicates $K_v1.1$ in the proliferation of MCF7 breast cancer cells; however, the involvement of $K_v1.6$ still cannot be ruled out. Similar mechanisms exist in other tissues, since downregulation of $K_v1.1$ expression by siRNA interference significantly reduced the proliferation of rat gastric mucosal epithelial cells, also measured by [^{3}H]-Thymidine incorporation (Wu et al. 2006).

The BK channels appear to have only a minor role in the normal proliferation of MCF7 cells, and blocking them induces only weak depolarization. However, their expression level and activity is cell cycle dependent, both peaking at the end of G1 phase (Ouadid-Ahidouch et al. 2004a). The relevance of this linkage to the cell cycle is unknown. However, BK expression may relate to the invasiveness of breast cancer cell lines. MDA MB 361 cells, with high levels of both *KCNMA1* mRNA and BK protein was considerably more invasive on a matrigel coated membrane that either MDA MB 231 or MCF7 cells, which both display much lower levels of the protein and mRNA. Untransformed mammary epithelial cell line MCF10A was not invasive and had very low levels of BK protein present (Khaitan et al. 2009).

Potassium Channels and Apoptosis

Interestingly, the K^+ channels have also been implicated as key regulators of apoptosis in many cell types (Wang 2004). Cell shrinkage is known to be an essential early stage in apoptosis (Vu et al. 2001). A number of studies have shown that K^+ currents determine the osmolarity of cells and therefore cell volume, and that cell shrinkage is attributable largely to K^+ efflux (Bortner and Cidlowski 1999; Gow et al. 2005; Hughes et al. 1997). These processes are tightly coupled during apoptosis in many cell lines including MDA MB 231 breast cancer cells (Gow et al. 2005; Vu et al. 2001).

In vitro studies have shown that K^+, at normal, nonapoptotic intracellular levels, directly inhibits apoptotic DNA fragmentation and caspase 3 activation in rat thymocytes (Hughes et al. 1997). In the same study, disrupting K^+ efflux in these cells by incubating them in medium containing high extracellular K^+ levels inhibited apoptosis and caspase 3 activation in response to apoptotic agents, suggesting that K^+ efflux is a necessary event in apoptosis.

The interaction between caspase activation, shrinkage and K^+ efflux is complex. Caspase activation correlates with K^+ efflux in lymphocytes treated with Fas apoptosis inducer or UV exposure (Vu et al. 2001). This laboratory found that caspase 3 and 8 inhibitors blocked DNA degradation in lymphocytes treated with Fas, but failed to prevent shrinkage and K^+ efflux, suggesting that K^+ efflux is an early cellular response which occurs before caspase activation (Bortner and Cidlowski 1999). In apparent contradiction to this, the same group later demonstrated that the polycaspase inhibitor z-VAD-fmk abrogated cell shrinkage and K^+ efflux, in addition to preventing DNA damage and

caspase activation in Fas-treated lymphocytes (Vu et al. 2001). However, it was less effective at preventing UV-induced apoptosis. They went on to show, using specific caspase inhibitors and mutants lacking individual caspase genes, that caspase 8 is required for Fas-induced cell shrinkage, K^+ efflux, and programmed cell death. This confirms the role of caspase 8 described in the literature (Medema et al. 1997). Correspondingly, caspase 9 has similar indispensible roles in UV induced apoptosis (Vu et al. 2001). These findings suggest that apoptotic K^+ efflux and caspase activation are tightly coupled but differentially regulated depending on the route of apoptosis induction.

In a number of tumor cell lines functional hERG K^+ channels are required for effective induction of apoptosis in response to H_2O_2, and H_2O_2 treatment increased the outward flow of K^+ (Wang et al. 2002). In this study, cells lacking functional hERG required much higher concentrations of H_2O_2 to induce apoptosis, and in cells with functional hERG, cotreatment with the hERG blocker dofetilimide caused a dramatic reduction in the number of apoptotic cells after H_2O_2 treatment. It is possible that this function of hERG relates to the relationship between the expression levels of this channel and the increasing sensitivity to anticancer drugs described by Chen et al. (2005). However, while the hERG channel is present in MCF7, this effect has not been investigated in these cells.

Expression of hERG is similarly required for tumor necrosis factor α (TNFα) induced apoptosis (1 and 10 ng/ml) (Wang et al. 2002). Interestingly, lower doses of TNFα (1 and 0.1 ng/ml), which were less effective at inducing apoptosis, also enhanced proliferation. Again, this effect was more pronounced in cells expressing hERG, but paradoxically was not affected by dofetilimide treatment. Fluorescent antibodies were then used to suggest that hERG recruits the TNF-receptor (TNFR1) to the plasma membrane. The TNFRs (TNFR1 and TNFR2) have complex roles in the regulation of both apoptosis and proliferation, which are incompletely understood (Baxter et al. 1999; Haider and Knofler 2009). In apoptosis TNFR1 activates caspase 3, triggering the caspase cascade in this manner. The TNFRs induce proliferation through the transcription factor NF-κB (Haider and Knofler 2009). In accordance with this, cells expressing hERG showed higher levels of NF-κB activity than cells lacking hERG expression, and that TNFα treatment induced a further increase in NF-κB activity (Wang et al. 2002). Interestingly, hERG has also been implicated in tumor proliferation (Pardo et al. 2005).

In addition to their roles in the proliferation of breast cancer, there is evidence to suggest that the channels $K_v1.1$ and $K_v1.3$ are involved in apoptosis in some cell lines also. One such study used the lymphocyte cell line CTTL-2, which is known to be deficient in K_v channels, and transfected them with a $K_v1.3$ expression vector or control expression vector (Szabo et al. 2008). It was found that the presence of $K_v1.3$ specifically on the mitochondrial membrane both amplified and accelerated the ability of the lymphocytes to induce apoptosis in response to a number of stimuli, including TNFα and staurosporine. They also demonstrated that overexpression of Bax triggered massive apoptosis in the $K_v1.3$-positive cells, but had no effect in cells lacking $K_v1.3$. Similarly, in rat retinal ganglions which constitutively express $K_v1.3$, blocking the channel with the relatively specific channel blockers agitoxin-2 or margatoxin greatly inhibited the ability of these cells to undergo apoptosis, and reduced the expression of the pro-apoptotic genes encoding caspase 3, caspase 9 and Bad, as determined by RT-PCR. Silencing the gene with siRNA had the same effect (Koeberle et al. 2010).

$K_v1.1$ has also been identified on the mitochondrial membrane in addition to the cytoplasmic membrane and, like $K_v1.3$, appears to have a role in the induction of apoptosis in lymphocytes (Szabo et al. 2008) and retinal ganglions (Koeberle et al. 2010). However, the mechanisms through which the two channels induce apoptosis may be different, as siRNA silencing of the $K_v1.1$ gene in rat retinal ganglions had no significant effect on the expression levels of caspase 3, caspase 9 and Bad (pro-apoptotic member of the Bcl2 family), but increased the levels of the antiapoptotic gene Bcl-xl (Koeberle et al. 2010). Neither $K_v1.1$ nor $K_v1.3$ knockdown was found to affect the levels of Bcl2 mRNA.

Silencing of the gene encoding $K_v1.2$ in rat retinal ganglions caused some reduction in the ability to undergo apoptosis, although this was to a much lesser extent than $K_v1.1$ or $K_v1.3$ silencing, and ablation of $K_v1.5$ was found to have no effect (Koeberle et al. 2010). The VGKCs are also involved in induction of apoptosis in pulmonary artery smooth muscle cells, where incubation with 4-AP before an apoptotic stimuli reduces apoptosis significantly, increases intracellular K^+ concentration, inhibits caspase activity and prevents mitochondrial cytochrome c release (Park et al. 2010). Breast cancer cells have been demonstrated to express a number of VGKCs at different levels to untransformed cells or normal tissue. Although the involvement of $K_v1.3$ in breast cancer cells is controversial, it has been reported in lower levels in cancerous breast tissue compared to normal tissue by one group (Brevet et al. 2008). In addition, Ouadid-Ahidouch et al. (2000) confirmed that the protein is not present in MCF7 cells. Absence or low levels of this K^+ channel may be related to the low levels of apoptosis seen in cancerous cells. However, surprisingly, the involvement of the VGKCs in the induction of apoptosis in breast cancer cells has not been investigated.

Isoflavones and Breast Cancer

A recent meta-analysis of epidemiologic studies has shown an inverse relationship between dietary soy, particularly the high levels of consumption seen in traditional Eastern Asian diets, and breast cancer risk (Trock et al. 2006). This reduction in risk is particularly strongly associated with premenopausal breast cancer (Lee et al. 2009), and ER-positive tumors (Suzuki et al. 2008).

It is widely agreed that the anticancer properties of soy are due to its high phytoestrogen content. Phytoestrogens are natural, plant metabolites with weak estrogenic activity, and a structure, summarized in Fig. 2, similar to mammalian estrogens of which E2 is the major form (Hwang et al. 2006; Kuiper et al. 1998). Many plants produce estrogenic compounds, but of the types consumed by humans, the isoflavone phytoestrogens found in soy, mostly genistein and daidzein have received the most scientific attention (Dixon 2004). In Eastern Asian individuals consuming a traditional high soy diet, serum levels of genistein and daidzein can reach concentrations of approximately 1 μM (Arai et al. 2000; Iwasaki et al. 2008). In comparison Western individuals, including UK women, who consume little or no soy, have serum concentrations closer to 1 nM (Grace et al. 2004; Verkasalo et al. 2001).

There are numerous animal and in vitro (cell culture) studies suggesting that high (μM) concentrations of isoflavones, including genistein and daidzein, inhibit the proliferation of breast cancer and induce apoptosis. However, low (nM) concentrations induce the proliferation of ER-positive breast cancer cells, such as MCF7 cells, in a similar manner, although to a lesser extent, than E2 (Hwang et al. 2006; Li et al. 2008; Maggiolini et al. 2001; Matsumura et al. 2005; Sakamoto et al. 2010; Shim et al. 2007). Isoflavones do not induce the proliferation of estrogen receptor negative cells (including MDA MB 231 cells) at any concentrations, and addition of ER antagonists such as tamoxifen to ER-positive cells prevents the isoflavones from inducing proliferation (Hwang et al. 2006; Maggiolini et al. 2001), suggesting that isoflavones act on proliferation through the estrogen receptors (ERs), and are acting as estrogen agonists.

Even in the presence of premenopausal E2 concentrations, high (μ) concentrations of isoflavones such as genistein and daidzein appear to reduce proliferation and induce cell death, at least partially reversing the E2-induced stimulatory effect on ER-positive breast cancer cell growth (Maggiolini et al. 2001; Peterson and Barnes 1996; Sakamoto et al. 2010; So et al. 1997; Zava and Duwe 1997). These studies tend to suggest that high levels of isoflavones, similar to those achieved in the serum of Eastern Asians consuming 'traditional' high-soy diets, can protect against breast cancer at premenopausal estrogen concentrations, by inducing apoptosis and inhibiting the estrogen-induced stimulation of proliferation in ER-positive breast cancer cells. This corresponds with the results of epidemiologic studies. There are numerous proposed mechanisms through which the protective effects of isoflavones may act, but it is possible that their effects on membrane potassium channels may play a key role.

Effects of Isoflavones on the Activity of Potassium Channels: A Breast Cancer Protective Mechanism?

Because of its nonspecific tyrosine kinase inhibitory actions, genistein is frequently utilized in studies investigating the regulation of K^+ channel activity. In this manner it had been determined that genistein, in concentrations ranging from 10 to 100 μM inhibits K^+ current through many channels in a number of excitable cardiac cells or

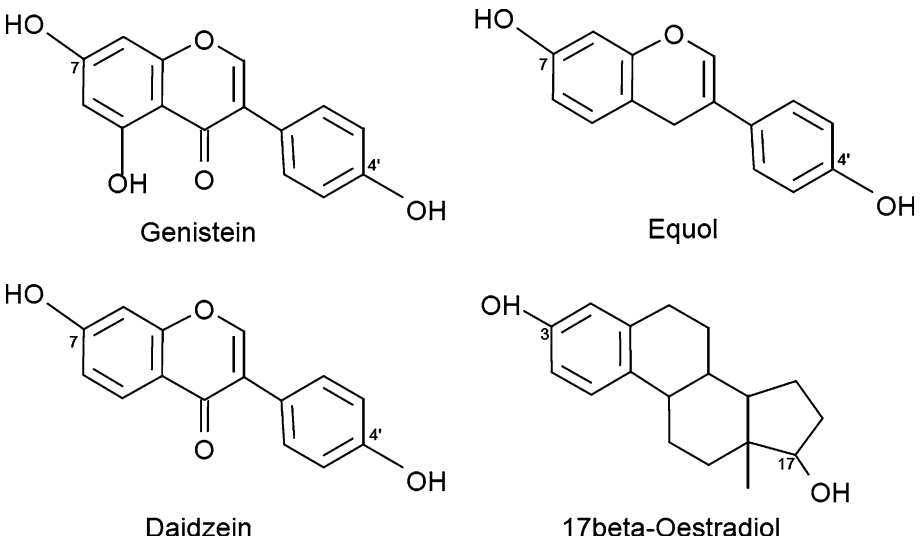

Fig. 2 Structure of the main isoflavones and 17β-estradiol

lymphocytes, with resting membrane potentials of around −70 mV (Cayabyab and Schlichter 2002; Chiang et al. 2002; Dong et al. 2010; Gao et al. 2004; Gierten et al. 2008; Missan et al. 2006; Teisseyre and Michalak 2005; Vaidyanathan et al. 2010). In most of these cases, the response to genistein or other protein tyrosine kinase (PTK) inhibitors has been very rapid (within seconds or minutes of treatment) arguing for a direct influence on signaling pathways or the channel proteins themselves, rather than changes in gene expression. Since many of these channels have roles in proliferation and apoptosis, the impact of K^+ channel inhibition by genistein may be relevant to the pro-proliferative or pro-apoptotic actions of this phytoestrogen. However to the authors' knowledge, no studies to date investigating the effects of phytoestrogens, including genistein, on K^+ channels in cancerous breast epithelial cells (resting membrane potential of around −20 mV) have been carried out. Genistein inhibits K^+ current through numerous channel proteins; however, only those known to be expressed in breast cancer cells or tissue will be discussed in this review.

hERG and Rat Homologue rERG

Using HEK293 cells stably transfected with hERG, it was found that the K^+ current through this channel was inhibited by 30 μM genistein (Zhang et al. 2008). Cotreatment with 1 mm orthovanadate (protein tyrosine phosphatase; PTP inhibitor) countered the suppression of current, signifying that hERG K^+ current inhibition by genistein is dependent upon its ability to inhibit PTK activity. Orthovanadate alone failed to have the opposite, current promoting effect suggesting that basal levels of TK-substrate phosphorylation may be saturated. Daidzein (PTK inactive analogue of genistein) treatment resulted in some current inhibition at higher doses, although this was much less pronounced, making it impossible to rule out the possibility that higher doses of genistein may have some direct channel blocking properties also.

Additionally, Zhang et al. (2008) demonstrated that the hERG current was inhibited by the selective PTK inhibitors AG556 and PP2 (both at 10 μM). These compounds inhibit EGFR and Src-family tyrosine kinase activity, respectively, and therefore implicate both kinases in the regulation of hERG. Whether genistein, AG556 and PP2 inhibit current through hERG in the same manner is not investigated. However, Western blots demonstrate that genistein, AG556 and PP2, at the doses described, each reduce phosphorylation of the channel protein in a manner antagonized by orthovanadate (Zhang et al. 2008). Again the PTP inhibitor alone had no effect on channel phosphorylation suggesting that under control conditions hERG phosphorylation is saturated.

Whole cell patch clamp recordings taken from MSL-9 cells derived from rat microglia identified a depolarization activated inwardly rectifying current that was fully and specifically blocked by 1 μM E-4031 treatment, suggesting that it was mediated by rERG (the rat homologue of hERG, 99% homology) (Cayabyab and Schlichter 2002). After 15 to 20 min of treatment with the broad spectrum PTK inhibitors lavendustin A or genistein (each at 50 μM) current amplitude was reduced to a significantly greater extent than the spontaneous rundown effect (35% and 60%, respectively). Daidzein (50 μM) had no significant effect. The same group demonstrated that treatment with the Src-selective PTK inhibitor herbimycin A for over 12 h reduced current amplitude by approximately 70%. This data indicates that rERG K^+ current is inhibited by broad and Src-specific PTK inhibitors. Using Western blots with anti-rERG and anti-phosphotyrosine antibodies, Cayabyab and Schlichter (2002) proceeded to show that rERG was constitutively tyrosine phosphorylated in these circumstances, and that 12 h of pretreatment with genistein (50 μM) or herbimycin A (3 μM) significantly reduced tyrosine phosphorylation of rERG, by 40% or 25%, respectively.

These studies together suggest that hERG, and its homologue rERG, are regulated by PTK activity, including by the EGFR and Src-related kinases, and that genistein treatment inhibits ERG K^+ current through its ability to inhibit a broad spectrum of PTK activity. No attempt has been made to distinguish between direct inhibition of PTK phosphorylation of the channel or upstream interactions with other signaling molecules. The precise mechanism of inhibition is not known in either case. The close homology between rERG and hERG makes it likely that the two channels are regulated in a similar manner, although caution must be used when making direct comparisons.

The hERG channel does not appear to have a role in the proliferation of breast cancer cells (Roy et al. 2008), so its inhibition by genistein is unlikely to directly relate to the growth inhibitory effects of this isoflavone. However, the doses of genistein used to inhibit hERG K^+ current are comparable to the range of doses used to inhibit proliferation and induce apoptosis in both ERα-positive and ER-negative breast cancer cell lines. This is paradoxical as previous evidence suggests that pharmacologic blockade or inability to express hERG impairs the ability of cells to induce apoptosis in response to stimuli (Wang et al. 2002). In addition, the doses used in these studies are higher than physiologically relevant serum concentrations of up to approximately 1 μM, although neither of the above groups has provided data for a dose-response or IC_{50} for genistein and hERG activity; thus, it is possible that lower doses are also effective or act differently. This makes the relevance of this data to the effects of phytoestrogens on breast cancer cells questionable. It would be of more value to

investigate the effect of genistein on the activity of hERG in breast cancer tissue or cell lines.

$K_v1.3$

As demonstrated by whole cell patch clamp, voltage-sensitive K^+ current amplitude was reduced to under 50% by 40 μM genistein in human T lymphocytes, collected from blood samples (Teisseyre and Michalak 2005). Current blockade by genistein was dose-dependent, with half-maximal blockage occurring in the concentration range between 10 and 40 μM. Current activation was also slower after genistein treatment. In these cells the VGKC current is carried predominantly by the $K_v1.3$ channel (Cahalan et al. 2001), and the current was completely blocked by addition of 5 mM 4-AP, suggesting that these channels may be the target of genistein inhibition in lymphocytes.

Teisseyre and Michalak (2005) also found that the current, after cotreatment with 10 μM genistein and 1 mM orthovanadate, was not significantly different to the current after genistein treatment alone. This suggests that in the case of $K_v1.3$ channels in human T lymphocytes, current inhibition by genistein occurs in a predominantly PTK-independent manner. $K_v1.3$ current was unaffected by treatment with 40 μM daidzein. In both T lymphocytes and breast cancer cells expressing $K_v1.3$, treatment with the VGKC blocker tetraethyl ammonium (TEA) inhibited proliferation, as measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and [^{3}H]-thymidine incorporation into DNA, respectively (Cahalan et al.2001; Jang et al. 2009). In both cases, the doses of TEA used were nontoxic.

The doses of genistein used in to inhibit current through $K_v1.3$ (10 to 40 μM) correspond with the range of doses known to inhibit proliferation and induce apoptosis in breast cancer cell lines. These doses are slightly higher than the physiologically relevant high serum level of 1 μM; however, as discussed above, differences in experimental conditions and media may account for incongruities between the IC_{50}s for genistein on K^+ channel activity and proliferation. Bearing this in mind it is possible that genistein may inhibit breast cancer proliferation via inhibition of the $K_v1.3$ channel. $K_v1.3$ is also involved in the induction of apoptosis in a number of cell lines, but there is no evidence to suggest that genistein treatment inhibits the induction of apoptosis. However, while this channel is expressed in many breast cancer tissues, it has not been detected in MCF7 cells (Ouadid-Ahidouch et al. 2000). It is interesting to note that $K_v1.3$ in human T lymphocytes was also inhibited in a similar manner by resveratrol, a phytoestrogen found in grapes and wine, with an IC_{50} value calculated to be 40.9 ± 5.0 μM (Teisseyre and Michalak 2006).

BK Channel

Resveratrol has been demonstrated to dose dependently stimulate outward BK and IK channel activity in vascular endothelial cells derived from a human umbilical cord, with an EC_{50} of 20 μM, using the whole cell patch clamp technique. This same group found that quercetin (30 μM) had no effect (Li et al. 2000). Resveratrol appeared to enhance channel activity by increasing the length of time that each channel was open for, rather than raising the conductance of individual channels.

Puerarin, the main isoflavone found in the root of the leguminous creeper Kudzu (*Pueraria lobata),* also potently and rapidly activated the BK channel in *Xenopus* oocytes, when applied to the cytoplasmic side of an excised cell membrane patch at negative potentials (Sun et al. 2007). With no added Ca^{2+}, the dose response curve for puerarin generated an EC_{50} of 12.6 nM. The activation of BK current was Ca^{2+}-dependent, and 10 μM Ca^{2+} treatment increased the amplitude of the current, but the combination of 10 μM Ca^{2+} and puerarin gave a lower EC_{50} of 0.8 nM, and indicated that calcium may facilitate BK activation by puerarin. Alternately, it could be argued that puerarin increases the Ca^{2+} sensitivity of the channel. Daidzein, a hydrolysate of puerarin, lacking a glycosyl residue at the 8 position, also increased the BK current, but to a lesser extent than puerarin, suggesting that the 8-glucosyl residue plays a role in the activation of the channel.

To the author's knowledge, the effect of genistein on the activity of the BK channel is not known. Although BK channel activity appears to be unrelated to the regulation of proliferation in breast cancer cell lines, there is evidence suggesting that it plays a role in invasiveness or metastasis (Khaitan et al. 2009). This may be a mechanism through which phytoestrogens exert some of their protective effects against breast cancer.

Limitations of Patch Clamp Methodology

Patch clamping is considered to be the gold standard method with which to measure ion currents as it allows the channels to be gated by physiologically relevant membrane potentials (Birch et al. 2004). However, there are a number of criticisms which can be leveled at each of these studies using it. Firstly, many of these experiments have looked in real time at the acute effects of compounds on channel activity, usually recording events occurring within 10 or 20 min of treatment. It is in the nature of the patch clamp methodology that membrane integrity begins to deteriorate after this time, as the cell dies. Pretreatment for longer periods would be required to occur in advance, and therefore longer-term effects on K^+ currents cannot be

measured in real time. The result is that only short-term effects are seen, which are more likely to reflect post-translational regulation, direct channel gating, and activation cascades, rather than changes in gene expression, translational regulation, or protein half-life.

Another limitation of patch clamping methodology is that it is unrealistic to compare the concentrations of compounds used for K^+ channel experiments with the concentrations known to regulate other cellular processes in vivo or in cell culture conditions, such as PTK inhibitory IC_{50} or doses which promote proliferation. This is due to the requirement for slightly different experimental conditions. Although every attempt is made to make the media for a patch clamp experiment as physiologically relevant as possible, in order to accurately measure currents, the serum normally added to cell culture media is absent, and various ion concentrations and pharmacologic agents are frequently manipulated to allow the experimenter to isolate individual currents. Also, the pipette solution which bathes the intracellular space needs to mimic the normal intracellular milieu as closely as possible, but it is at best a compromise. This regrettably makes it impossible to make direct links between K^+ flux effects and alterations in the proliferative or apoptotic activity of cells. However, as discussed, studies which have silenced specific channel genes, or transfected them into cells known to not normally express it, add strength to the arguments here.

Finally, in numerous instances discussed above, doses of PTK inhibitors have been used in vast excess of their IC_{50} to guarantee PTK inhibition. They may be cytotoxic or generate nonspecific effects at these high doses. Validation of the relevance of the doses used is required, possibly by comparison of the patch clamp results with results of other methods of assessing K^+ movement, such as use of radiolabelled rubidium or fluorescent probes, such as potassium-binding fluorescent indicator (PBFI), although these methods too are not without limitations.

Conclusion

Potassium channels appear to have a central role in the regulation of proliferation and apoptosis in breast cancer, in both tissue samples and breast cancer cell lines such as MCF7. Their protein and expression levels frequently depend upon the stage and tumorigenicity of the cancer. In a number of cases, the same channels are involved in both proliferation enhancement and the induction of apoptosis.

Genistein, through its ability to inhibit PTK activity and other mechanisms, has been shown inhibit the activity of a number of K^+ channels, including, although not limited to hERG and $K_v1.3$. Most of these studies have been carried out using excitable cardiac cells or lymphocytes, and the effect of genistein on the K^+ channels in cancerous breast epithelial cells is not yet known. Since many of these channels have roles in proliferation and apoptosis in breast cancer cells, the impact of K^+ channel inhibition by genistein may be relevant to its protective actions against breast cancer. Although further investigation is required, K^+ channel activity shows some promise as a pharmacologic target against breast cancer and may represent a mechanism through which phytoestrogens act on these cells.

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