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Themed Section: The pharmacology of TRP channels

REVIEW

New insights into pharmacological tools to TR(i)P cancer up

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The aim of this review is to address the recent advances regarding the use of pharmacological agents to target transient receptor potential (TRP) channels in cancer and their potential application in therapeutics. Physiologically, TRP channels are responsible for cation entry (Ca^{2+}, Na^{+}, Mg^{2+}) in many mammalian cells and regulate a large number of cellular functions. However, dysfunction in channel expression and/or activity can be linked to human diseases like cancer. Indeed, there is growing evidence that TRP channel expression is altered in cancer tissues in comparison with normal ones. Moreover, these proteins are involved in many cancerous processes, including cell proliferation, apoptosis, migration and invasion, as well as resistance to chemotherapy. Among the TRP superfamily, TRPC, TRPV, TRPM and TRPA1 have been shown to play a role in many cancer types, including breast, digestive, gliomal, head and neck, lung and prostate cancers. Pharmacological modulators are used to characterize the functional implications of TRP channels in whole-cell membrane currents, resting membrane potential regulation and intracellular Ca^{2+} signalling. Moreover, pharmacological modulation of TRP activity in cancer cells is systematically linked to the effect on cancerous processes (proliferation, survival, migration, invasion, sensitivity to chemotherapeutic drugs). Here we describe the effects of such TRP modulators on TRP activity and cancer cell phenotype. Furthermore, the potency and specificity of these agents will be discussed, as well as the development of new strategies for targeting TRP channels in cancer.

LINKED ARTICLES

This article is part of a themed section on the pharmacology of TRP channels. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2014.171.issue-10

Non-standard abbreviations

AMPK, AMP-activated protein kinase; 2-APB, 2-aminoethoxydiphenylborate; ATRA, all-trans retinoic acid; CAI, carboxyamidotriazole; CBD, cannabidiol; CIPN, chemotherapy-induced peripheral neuropathy; GBM, glioblastoma multiforme; 20-GPPD, 20-O-β-D-glucopyranosyl-20(S)-protopanaxadiol; NSCLC, non-small cell lung cancer; ROS, reactive oxygen species; RQ, RQ-00203078 (TRPM8 antagonist); SOCE, store-operated calcium entry; TRP, transient receptor potential; TRPA, transient receptor potential ankyrin; TRPC, transient receptor potential canonical; TRPM, transient receptor potential melastatin; TRPML, transient receptor potential mucolipin; TRPP, transient receptor potential polycystin; TRPV, transient receptor potential vanilloid

General context

Cancer is one of the leading causes of death in the world. Despite developments in early detection and additions to the therapeutic arsenal, there is still a lack of effective therapeutic strategies. Therefore, there is a need to discover new biomarkers and therapeutic targets to improve clinical outcomes for cancer patients.

Tumour progression results from alterations of physiological processes such as cell proliferation, apoptosis, migration, invasion and angiogenesis. These processes are under the control of calcium homeostasis, and the TRP (transient receptor potential) cation channels (Alexander *et al.*, 2013) contribute to changes in intracellular calcium concentration (Nilius *et al.*, 2007). About thirty TRPs have been identified to date, and they are classified in seven different families: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPML (mucolipin), TRPP (polycystin), TRPA (ankyrin transmembrane protein) and TRPN (NompC-like) (Venkatachalam and Montell, 2007). TRP channels play a crucial role in a variety of physiological and pathological processes (Nilius *et al.*, 2007; Wu *et al.*, 2010), and the TRPC, TRPM and TRPV families have mainly been correlated with malignant growth and progression. Indeed, recent findings demonstrate that TRP channels are involved in the regulation of proliferation, differentiation, apoptosis, angiogenesis, migration and invasion during cancer progression, and that the expression and/or activity of these channels is altered in cancers (Prevarskaya *et al.*, 2007; 2011; Wu *et al.*, 2010). It has been proposed that the expression pattern of TRP channels may serve a tool for diagnostics and/or prognostics in cancer. For example, TRPV6 and TRPM8 have been proposed as tumour progression markers in the outcome of prostate cancer (Gkika and Prevarskaya, 2011), TRPC6 may be a novel therapeutic target in oesophageal carcinoma (Ding *et al.*, 2010a), and TRPV6 and TRPM7 may serve as markers associated with poor outcome in metastatic breast cancer (Ouadid-Ahidouch *et al.*, 2013).

Novel molecules targeting TRP calcium channels (in particular, TRPA1, TRPV1, TRPM8, and TRPC6) are currently being studied in clinical trials or are candidates for future clinical evaluation in the management of respiratory diseases (Preti *et al.*, 2012). Moreover, thermo-TRP channels, including TRPV1, TRPV2, TRPV3, TRPV4, TRPM8 and TRPA1, are known to be regulated by extracellular modulators released by damaged or inflamed tissues, and several antagonists of the channels are in advanced-stage clinical trials as potential analgesics (Vay *et al.*, 2012). With regard to therapies targeting calcium homeostasis in cancer, carboxyamidotriazole (CAI) was one of the first targeted therapies to reach clinical trial (Kohn *et al.*, 1997). CAI is a synthetic calcium influx inhibitor with anti-angiogenic, anti-proliferative and anti-metastatic properties *in vitro,* but published trials of CAI in renal cells, glioblastoma multiforme and non-small cell lung cancer did not show improvement in survival or quality of life (Stadler *et al.*, 2005; Mikkelsen *et al.*, 2007; Johnson *et al.*, 2008). However, a combination of CAI and paclitaxel, a standard chemotherapy agent, has enhanced anti-tumour activity in solid tumours, particularly in ovarian cancer, since CAI exposure potentiates the clinical activity of paclitaxel (Azad *et al.*, 2009). As TRP channels may be new promising markers for diagnosis and chemotherapy in several cancers (Santoni and Farfariello, 2011; Ouadid-Ahidouch *et al.*, 2013), targeting TRP channels may constitute a new pharmacological approach for cancer therapy in the future.

In this review, we describe the TRP channels from the point of view of pharmacological targets in cancer, and the molecular mechanisms by which the inhibitors affect proliferation, migration and invasion of cancer cells.

TRPC targeting in cancer

Seven mammalian TRPC proteins (TRPC1–7) have been identified. Based on their sequence homology, these channels can be divided into three subgroups: C1/C4/C5, C3/C6/C7, and C2, which is a pseudogene in humans (Wu *et al.*, 2010). TRPC channels allow Ca^{2+} and Na^{+} entry, and they are expressed in many mammalian cell types and involved in many human diseases including cancer (Bon and Beech, 2013). It has been proposed in a number of cell models that TRPC channels may be involved in store-operated Ca^{2+} entry (SOCE) by interacting with Orai1 and STIM1 (Berna-Erro *et al.*, 2012). In cancer cells, the role of TRPC channels has been linked to cell proliferation, survival and tumour growth (Santoni and Farfariello, 2011; Shapovalov *et al.*, 2011). Several studies have shown that TRPC channels are also important for chemotactic migration (Bomben *et al.*, 2011). Pharmacological strategies to target TRPC channels have been used in several cancer cell models. These results are described below and summarized in Table 1.

Pharmacological approaches to target TRPC in cancer

Activation or blockade of TRPC channels generally induces the same effect, cell growth arrest. Thus, modulation of TRPC channels can have the opposite effect. For example, activation of Ca^{2+} influx through TRPC channels by 20-*O*-β-D-glucopyranosil-20(*S*)-protopanaxadiol (20-GPPD), a metabolite of ginseng saponin, induced apoptosis in colon cancer cells (Hwang *et al.*, 2013). Specifically, in CT-26 murine colon cancer cells, application of 20-GPPD (10 μM) for 24 h induced apoptotic cell death, with an accumulation of cells in the sub-G1 phase. Furthermore, 20-GPPD increased AMP-activated protein kinase (AMPK) phosphorylation in a time-dependent manner, leading to AMPK activation and cell viability reduction. The increased AMPK activation induced by 20-GPPD was triggered by an increase in cytosolic [Ca^{2+}] entry through TRPC channels. Indeed, Ca^{2+} influx induced by 20-GPPD is sensitive to the TRPC non-selective blockers Gd^{3+} (100 μM) and SKF96365 (10 μM), whereas the TRPV1 antagonist capsazepine (20 μM) has no effect. Whether the effect of 20-GPPD on TRPC channels is direct has not yet been clearly established.

In non-small cell lung cancer (NSCLC), one study found that TRPC1, C3, C4 and C6 expression was correlated with tumour differentiation grade (Jiang *et al.*, 2013). In this study, all-trans retinoic acid (ATRA), a chemopreventive agent as well as a potent cell differentiation inducer, was used. Treatment of A549 cells with ATRA (1 μM) for 96 h increased TRPC3, C4 and C6 expression and enhanced Ca^{2+} influx. Moreover, A549 cell proliferation was more sensitive to the TRPC non-specific blocker 2-APB (EC_{50} = 87.9 μM) following chronic application of ATRA (Jiang *et al.*, 2013). However, ATRA showed no direct effect on TRPC channel function, and the increased Ca^{2+} influx was probably due to the up-regulation of the TRPC gene.

TRPC channels (TRPC1, 3, 4 and 5) are expressed in glioma cell lines and acute patient-derived tissues, suggesting the presence of heteromeric channels in gliomas (Bomben and Sontheimer, 2008). In the glioma cell line D54MG (grade

Table 1

Pharmacological modulation of TRPC channels in cancer cells

Pharmacological agent	TRP target	Cancer type	Effect	Mechanism	References
20-GPPD (10 μM)	TRPC activation?	Colon	Cell apoptosis	Ca^{2+} entry ↑	Hwang *et al.*, 2013
ATRA (1 μM)	TRPC3, C4, C6 up-regulation	NSCLC	Cell proliferation more sensitive to TRPC blockers	Ca^{2+} entry ↑	Jiang *et al.*, 2013
SKF96365 (25 μM) 2-APB (100 μM)	TRPC1, C3, C4, C5 blockade	GBM	Cell growth arrest and multinucleation	Em hyperpolarization	Bomben and Sontheimer, 2008
SKF96365 (25 μM) 2-APB (100 μM) MRS1845 (25 μM)	TRPC1 blockade	GBM	Cell growth arrest and multinucleation	Store-operated Ca^{2+} entry ↓	Bomben and Sontheimer, 2010
SKF96365 (25 μM) 2-APB (100 μM) MRS1845 (25 μM)	TRPC1 blockade	GBM	Cell migration ↓	Store-operated Ca^{2+} entry ↓	Bomben *et al.*, 2011
SKF96365 (10 μM)	TRPC6 blockade	Gastric, oesophageal	Cell growth arrest	Ca^{2+} entry ↓	Cai *et al.*, 2009; Shi *et al.*, 2009

IV), small linear voltage-independent currents that were sensitive to the non-specific TRPC inhibitors SKF96365 (25 μM) and 2-APB (100 μM) were recorded. Inhibition of TRPC channels further induced a membrane hyperpolarization, indicating that TRPC channel currents contribute to resting membrane potential in glioma cells. Moreover, chronic application of the TRPC inhibitors SKF96365 and 2-APB diminished cell proliferation without inducing cell mortality (Bomben and Sontheimer, 2008). This treatment also induced glioma cell accumulation in the G2/M phase following TRPC blockade, and led to an increase in mean cell volume and size, indicating an impairment of cytokinesis (Bomben and Sontheimer, 2008).

The further use of a shRNA strategy highlighted that the TRPC1 channel is essential for cell proliferation in the most frequent class of malignant primary brain tumour, glioblastoma multiforme (GBM) (Bomben and Sontheimer, 2010). Use of shRNA against TRPC1 almost completely eliminated the SKF-sensitive currents, SOCE and glioma proliferation, indicating that TRPC1 is, at least, an essential subunit for the function of TRPC in GBM. However, the ability of TRPC1 subunits to co-assemble in homotetramers is still debatable; whereas TRPC1 can form heteromeric channels with TRPC4 and TRPC5. The I:V relationship recorded for heteromers displays properties distinct from those for TRPC4 or TRPC5 monomers. Moreover, the unitary conductance of single heteromeric channels is also different from that for TRPC4 and TRPC5 monomers. Until now, TRPC1 was considered a component of different heteromeric TRP complexes.

One study has shown TRPC1 to regulate the glioma chemotaxis induced by epidermal growth factor (EGF) (Bomben *et al.*, 2011). This study showed that pharmacological blockade of TRPC1 using SKF96365 (25 μM), 2-APB (100 μM) or MRS1845 (100 μM), or TRPC1 silencing using shRNA inhibited EGF-induced chemotactic migration but not basal migration in GBM. Moreover, the localization of TRPC1 in lipid rafts is essential for TRPC function (SKF-sensitive current and SOCE) as well as for EGF-induced chemotaxis (Bomben and Sontheimer, 2010). It was also demonstrated that TRPC1 inhibition using 2-APB or siRNA resulted in significantly attenuated adhesive and invasive abilities of nasopharyngeal cancer cells, suggesting that TRPC1 can modulate metastasis in nasopharyngeal carcinoma (He *et al.*, 2012).

TRPC6 channels are essential for the G2/M phase transition in gastric and oesophageal cancers (Cai *et al.*, 2009; Shi *et al.*, 2009). In these studies, the non-specific pharmacological agent SKF96365 (10 μM) was used to block TRPC6 channels, leading to cell growth arrest and accumulation in the G2/M phase. The specific role of TRPC6 channels was confirmed by the heterologous expression of a dominant negative of TRPC6 (DNC6) in cancer cells. The regulation of cancer cell proliferation by TRPC6 involved an elevation of $[Ca^{2+}]_i$ that is essential for G2/M phase transition in gastric and oesophageal cancers.

Limitations in targeting TRPC channels in cancers

The main difficulty in targeting TRPC channels is the lack of pharmacological agents that specifically target a TRPC subtype. Indeed, in most studies on cancer cells, non-specific blockers such as SKF96365, 2-APB and MRS1845 were used to inhibit TRPC functions, including membrane current and SOCE, at a concentration in the micromolar range. SKF96365 belongs to the family of phenylethylimidazoles, which are used as non-specific inhibitors of TRPC Ca^{2+} channels. However, these compounds are also known to block voltage-gated Ca^{2+} channels (Singh *et al.*, 2010). The organoborane 2-APB is also widely used as a pharmacological modulator of TRP channels. It blocks TRPC and TRPM channels while it stimulates TRPV channels (Hu *et al.*, 2004). Moreover, 2-APB was initially identified as a membrane-penetrating inhibitor of $Ins(1,4,5)P_3$-induced Ca^{2+} release (Maruyama *et al.*, 1997). Dihydropyridines like *N*-propargylnifrendipine (MRS1845) are known as a class of potent and selective blockers of voltage-gated L-type Ca^{2+} channels. MRS1845 has also been

described as a potent inhibitor of SOCE (Harper *et al.*, 2003) and has been used as a pharmacological tool to assess the role of TRPC channels in GBM (Bomben and Sontheimer, 2010; Bomben *et al.*, 2011). Therefore, the use of this pharmacological approach to target TRPC in pathologies, and particularly in cancer, is still far from ideal in preclinical studies because none of the pharmacological agents used to target TRPC in cancer seem to be highly potent and specific. The use of cell transfection with shRNA or with a dominant negative is required to specifically target the TRPC channel subunit that is essential for cancer progression. Thus the development of new pharmacological tools targeting TRPC channels is under investigation, as already highlighted in the recent review of Bon and Beech (Bon & Beech, 2013).

Moreover, TRPCs are known to form heteromers in a wide range of cell types. The knockdown of one specific TRPC subtype could be compensated for by other TRPCs or other Ca^{2+} entry mechanisms such as Orai channels. Thus, the complex structure of TRPC tetramers and the multiplicity of Ca^{2+} transporters that could compensate for a TRPC deficiency in the cells increase the difficulty of specifically targeting particular TRPC channels in malignant progression.

TRPV targeting in cancer

The TRPV (vanilloid) family is composed of six members (TRPV1–6) that have been identified in mammals. They have been divided into two subgroups based on their sequence homology and Ca^{2+} selectivity: V1/V2/V3/V4 and V5/V6 (Wu *et al.*, 2010). The TRPV1–4 subgroup is weakly Ca^{2+}-selective and highly sensitive to high temperatures. These channels are able to initiate sensory nerve impulses following the detection of chemical and thermal stimuli, and they are called 'thermo-TRP channels'. The role of these TRPV channels has been well studied with regard to pain. Indeed, TRPV1 and TRPV3 channels have been suggested as analgesic targets, and some TRPV1 and TRPV3 antagonists have already advanced to clinical trials (Moran *et al.*, 2011; Brederson *et al.*, 2013). In cancer, TRPV1 channel has been proposed as a pharmacological target in pain induced by cancer (Prevarskaya *et al.*, 2007). In contrast, TRPV5 and TRPV6 channels are highly Ca^{2+}-selective and not heat-sensitive. Physiologically, they allow Ca^{2+} entry in non-excitable cells and prevent Ca^{2+} overload by a Ca^{2+}-dependent inactivation (Hoenderop *et al.*, 2005). In normal tissues, TRPV5 and TRPV6 channels are mainly expressed in apical membranes of various epithelia, including the kidney, intestine, stomach and pancreas as well as the brain, bone and placenta (Vennekens *et al.*, 2008). TRPV6 channels are overexpressed in prostate cancer, where they have been suggested as oncogenic ion channels (Lehen'kyi *et al.*, 2012). The use of pharmacological agents to modulate TRPV in cancer will be described in detail below and summarized in Table 2.

TRPV1

TRPV1 expression can be decreased or increased, depending on cancer type and tumour stage (Santoni and Farfariello, 2011). TRPV1 modulators more widely used in cancer are the activator capsaicin and the antagonist capsazepine. In the human androgen-independent prostate cancer cell line PC-3, capsaicin (at 5 μM) induced a $[Ca^{2+}]_i$ increase that was antagonized by 1 μM capsazepine (Sanchez *et al.*, 2006). Moreover, 20 μM capsaicin inhibited DNA synthesis and increased the

Table 2

Pharmacological modulation of TRPV channels in cancer cells

Pharmacological agent	TRP target	Cancer type	Effect	Mechanism(s)	References
Capsaicin (IC_{50} 20 μM)	TRPV1 activation?	Prostate	Cell growth arrest and apoptosis	$[Ca^{2+}]_i$ ↑, ROS generation, ΔΨm dissipation, caspase-3 activation	Sanchez *et al.*, 2006
Capsaicin (50 μM)	TRPV1 activation	GBM	Cell apoptosis	Ca^{2+} entry	Amantini *et al.*, 2007
Capsaicin (IC_{50} 80 μM)	TRPV1 activation	Urothelial	Cell growth arrest and apoptosis	$[Ca^{2+}]_i$ ↑, ΔΨm dissipation, caspase-9 and -3 activation	Amantini *et al.*, 2009; Caprodossi *et al.*, 2011
Arvanil (50 nM)	TRPV1 activation	Astrocytoma	Cell apoptosis	ER stress	Stock *et al.*, 2012
Cannabidiol (IC_{50} 22.2 μM)	TRPV2 activation	GBM	Cell apoptosis (> 25 μM) and sensitization to cytotoxic chemotherapy (<10 μM)	Ca^{2+}-entry and drug uptake	Nabissi *et al.*, 2013
Soricidin derived peptides (SOR-C13 IC_{50} 14 nM, SOR-C27 IC_{50} 65 nM)	TRPV6 binding and blockade	Ovarian or prostate	Cell proliferation ↓; TRPV6-rich tumour labelling	$[Ca^{2+}]_i$ ↓	Bowen *et al.*, 2013
Capsaicin (50 μM)	TRPV6 activation	Gastric	Cell apoptosis	Ca^{2+}-entry, p53 stabilization, JNK activation	Chow *et al.*, 2007

number of apoptotic bodies. However, addition of 20 μM capsazepine was not able to reduce capsaicin-induced apoptosis but induced apoptosis in a similar manner. Both capsaicin and capsazepine increase the production of reactive oxygen species (ROS) as well as ΔΨm dissipation, suggesting that oxidant stress induced by vanilloids in PC-3 cells is independent of an effect of TRPV1. Both capsaicin and capsazepine further induced the activation of caspase-3 and the reduced tumour growth *in vivo*. Collectively, these data suggested that vanilloids could be used as promising pharmacological tools against hormone-refractory prostate cancer, but the importance of TRPV1 channels as a potential target is still unclear (Sanchez *et al.*, 2006).

However, TRPV1 has been identified as the main target of capsaicin-induced apoptosis in glioma (Amantini *et al.*, 2007). In the glioma cell line U373, capsaicin (50 μM) increased $[Ca^{2+}]_i$ and induced p38 MAPK activation, ΔΨm dissipation and caspase-3 activation, leading to cell apoptosis. All of these capsaicin-induced apoptosis effects were reversed following capsazepine treatment. Interestingly, TRPV1 expression was inversely correlated with grade in GBM suggesting that TRPV1 may negatively control cancer progression. Amantini *et al.* suggested that the loss of TRPV1 in high-grade GBM may represent a mechanism by which cancer cells can evade antiproliferative and pro-apoptotic signals (Amantini *et al.*, 2007). Additionally, capsaicin promotes Fas/CD95-mediated apoptosis of urothelial cancer cells (Amantini *et al.*, 2009). Capsaicin reduced in a dose-dependent manner (IC_{50} 80 μM) the proliferation of the human well-differentiated low-grade papillary RT4 urothelial cancer cell line. Moreover, capsaicin induced the up-regulation of pro-apoptotic genes as well as TRPV1-fas/CD95 receptor clustering and activation, which trigger both extrinsic and intrinsic mitochondrial-dependent pathways. Importantly, all of these effects were reversed by capsazepine (10 μM). Amantini *et al.* found that, as for gliomas, TRPV1 expression decreased in invasive urothelial cancers, with a complete loss of TRPV1 in high-grade cancers. Moreover, capsaicin induced a more aggressive gene phenotype and invasiveness in urothelial cancer cells lacking TRPV1 (Caprodossi *et al.*, 2011).

Importantly, Stock *et al.* showed that neural precursor cells induced cell death in high-grade astrocytomas by releasing endovanilloids that activate TRPV1 channels in cancer cells (Stock *et al.*, 2012). Cell death induced by TRPV1 activation in high-grade astrocytomas is dependent on endoplasmic reticulum (ER) stress due to the expression of TRPV1 in the ER membrane. Moreover, cell death in TRPV1-dependent high-grade astrocytomas can be reversed by capsazepine treatment. The synthetic, blood-barrier-permeable vanilloid arvanil (50 nM) has therapeutic effects on experimental high-grade astrocytomas, as it strongly reduces the size of astrocytomas and induces TRPV1-dependent cell death. Furthermore, treatment with arvanil prolonged survival in a cohort of immunodeficient mice implanted with a culture of human high-grade astrocytomas (Stock *et al.*, 2012).

The TRPV1 antagonist capsazepine has also been studied as a potential pharmacological tool in cancer. Indeed, 50 nM capsazepine sensitizes colorectal cancer cells to apoptosis induced by the TNF-related apoptosis-induced ligand (TRAIL) (Sung *et al.*, 2012). Capsazepine induced TRAIL receptor expression that could potentiate the use of cancer therapy based on TRAIL. However, in this latter study, up-regulation of TRAIL receptors by capsazepine seems to be TRPV1-independent. Moreover, capsaicin and capsazepine were used to highlight the importance of TRPV1 channels as Ca^{2+} channels, mediating migration of human hepatoblastoma (HepG2) cells (Waning *et al.*, 2007).

TRPV1 channels have also been shown to be sensitive to endocannabinoids, which suppress cell invasion. Indeed, Ramer *et al.* showed that low concentrations of R(+)-methanandamide (0.1 μM) decreased the cell invasion through Matrigel and this effect was reversed by a TRPV1 antagonist (capsazepin 1 μM) in human cervical cancer cells (HeLa, C33A) as well as in human lung carcinoma cells (A549) (Ramer and Hinz, 2008). This suggests that TRPV1 activity could contribute to the anti-invasive action of R(+)-methanandamide. Interestingly, the anti-invasive properties of cannabidiol were confirmed *in vivo* in a mouse model of lung metastasis (Ramer *et al.*, 2010). It is important to mention here the controversy regarding the potential use of capsaicin for cancer treatment. While capsaicin has been shown to induce apoptosis and cell cycle arrest in some cancer cell lines, other studies have highlighted its involvement in mutagenesis and carcinogenesis. These ambiguous results are extensively discussed elsewhere (Bode and Dong, 2011) and suggest that the cellular effects of capsaicin are far from being fully understood. Thus, it is premature to propose the use of capsaicin for cancer treatment. Moreover, another inconvenience associated with TRPV1 targeting in disease and particularly in cancer is the induction of undesirable on-target side effects. Indeed, TRPV1 is involved in body thermoregulation and the use of antagonists causes hyperthermia (Romanovsky *et al.*, 2009).

TRPV2

Nabissi *et al.* showed that cannabidiol (CBD), a cannabinoid compound that lacks the unwanted psychotropic effects associated with Δ^9-tetrahydrocannabinol, increased drug uptake and potentiated cytotoxic activity in GBM when co-administered with cytotoxic agents (Nabissi *et al.*, 2013). Thus, CBD acts as a TRPV2-selective activator by enhancing Ca^{2+} influx in U87MG cells with an EC_{50} of 22.2 μM. It was concluded that CBD could be used as a promising therapeutic agent against GBM because it potentiates the cytotoxicity of chemotherapeutic agents such as temozolomide, carmustine and doxorubicin by increasing the drug uptake in a TRPV2-dependent manner (Nabissi *et al.*, 2013). Moreover, TRPV2 has been shown to promote both *in vitro* and *in vivo* differentiation of glioblastoma stem-like cells, leading to a decreased proliferation rate (Morelli *et al.*, 2012).

In contrast, it has been shown that endogenous lysophospholipids, including lysophosphatidylcholine and lysophosphatidylinositol (both at 10 μM), enhance the migration of human prostate cancer (PC-3) cells via Ca^{2+} influx through TRPV2 channels by promoting channel translocation to the membrane (Monet *et al.*, 2009). Similarly, adrenomedullin, at a concentration of 200 nM, promotes migration and invasion of prostate cancer (PC-3) and urothelial cancer (T24/83) cells (Oulidi *et al.*, 2013). Interestingly, TRPV2 modulates adrenomedullin stimulation of cell migration and invasion by facilitating channel translocation to the membrane and

increasing the basal $[Ca^{2+}]_i$. Finally, *trpv2* transcripts were up-regulated in advanced stages of bladder cancer.

Therefore, the role of TRPV2 in cancer is still debatable. While it has been described as a regulator of stem-like cell differentiation and chemotherapeutics uptake in GBM, TRPV2 is also associated with the metastatic status of prostate and bladder cancers, where it stimulates cell migration and invasion. This limits the potential of using TRPV2 channels as pharmacological targets.

TRPV6

TRPV6 channels are overexpressed in a wide range of malignant cancers (Lehen'kyi *et al.*, 2012). Soricidin and its derivatives (SOR-C13 and SOR-C27) have been reported to inhibit TRPV6-dependent Ca^{2+} uptake and to bind TRPV6 in ovarian cancer cells with high affinity (Bowen *et al.*, 2013). Soricidin is a 54-amino acid peptide found in the paralytic venom of the northern short-tailed shrew *Blarina brevicauda*. Recently, Bowen *et al.* described the use of the TRPV6-binding properties of SOR-C13 and SOR-C27 to target human ovarian tumours in a xenograft mouse model (Bowen *et al.*, 2013). The TRPV6 specificity of soricidin derivatives may provide a way of delivering diagnostic and therapeutic reagents directly to TRPV6-rich tumours. Moreover, TRPV6 has been shown to mediate capsaicin-induced apoptosis in gastric cancer cells (Chow *et al.*, 2007). Indeed, capsaicin at 50 μM induced apoptosis in the human gastric cancer AGS cells without causing necrosis. Interestingly, AGS cells were more sensitive to capsaicin-induced apoptosis than normal gastric cells. Furthermore, capsaicin increased TRPV6 expression in a time-dependent manner. The capsaicin-induced TRPV6 expression and AGS apoptosis were prevented by pretreatment with the capsaicin antagonist capsazepine. The mechanism involved in the capsaicin-induced AGS apoptosis was an increase in Ca^{2+} influx via TRPV6 channels. Moreover, capsaicin induced apoptosis by stabilization of p53 through JNK activation.

TRPM targeting in cancer

The TRPM family is composed of eight members (TRPM1–8) and is divided into three subgroups based on their amino-acid homology: M1/M3, M4/M5, and M6/M7; TRPM2 and TRPM8 have low sequence homology and are not included in a subgroup (Wu *et al.*, 2010). TRPM1 has been discovered to be a tumour gene suppressor in melanoma (Duncan *et al.*, 1998), and TRPM1 and TRPM3 form a constitutively active Ca^{2+}-permeable non-selective cation channel. TRPM2 channels contain an ADP pyrophosphatase domain in their *C*-terminus, that binds to ADP ribose and hydrolyses it. Moreover, TRPM2 channels are also activated by oxidative stress, and thus are considered metabolic as well as intracellular oxidation sensors. TRPM4 and TRPM5 are monovalent-selective ion channels that are activated by intracellular Ca^{2+}. TRPM5 channels are expressed in taste receptor cells, while TRPM4 channels are ubiquitous. TRPM6 and TRPM7 channels are fused with a functional serine/threonine kinase located in their *C*-terminus. These channels have both ion channel and kinase activities. They allow the entries of Mg^{2+} and Ca^{2+} and the outflow of K^+. TRPM6 channels are expressed in kidney and intestine epithelia, where they are implicated in Mg^{2+} re-absorption. TRPM7 channels are ubiquitous but expressed at a low level in most tissues. However, TRPM7 is overexpressed in many cancers, including breast (Guilbert *et al.*, 2009; 2013; Middelbeek *et al.*, 2012) and pancreas (Rybarczyk *et al.*, 2012). TRPM8 channels are Ca^{2+}-permeable channels that are activated by cold (8–28°C) and cooling compounds like menthol and icilin. TRPM8 channels are widely expressed, but they have been proposed as a potential prognostic and diagnostic biomarker in androgen-dependent prostate cancer (Zhang and Barritt, 2006). The pharmacological agents that modulate TRPM in cancer are summarized in Table 3.

Table 3

Pharmacological modulation of TRPM channels in cancer cells

Pharmacological agent	TRP target	Cancer type	Effect	Mechanism	References
Waixenicin A (10 μM)	TRPM7 blockade	Leukaemia	Cell proliferation ↓	Mg^{2+}-dependent block of TRPM7	Zierler *et al.*, 2011
Waixenicin A (50 μM)	TRPM7 blockade	Breast and gastric	Cell proliferation ↓	TRPM7 current ↓	Kim *et al.*, 2013
Ginseroside Rd (500 μM)	TRPM7 blockade	Gastric	Cell growth and survival ↓	TRPM7 current ↓	Kim *et al.*, 2013
BCTC (10 μM)	TRPM8 blockade	Prostate	Cell proliferation ↓	Cold-activated $[Ca^{2+}]_i$ ↓	Valero *et al.*, 2011
Clotrimazole (10 μM)	TRPM8 blockade	Prostate	Cell proliferation ↓	Cold-activated $[Ca^{2+}]_i$ ↓	Valero *et al.*, 2011
DD01050 (10 μM)	TRPM8 blockade	Prostate	Cell proliferation ↓	Cold-activated $[Ca^{2+}]_i$ ↓	Valero *et al.*, 2011
AMTB (10 μM)	TRPM8 blockade	Prostate	Cell proliferation ↓	Not tested	Valero *et al.*, 2012
JNJ41876666 (10 μM)	TRPM8 blockade	Prostate	Cell proliferation ↓	Not tested	Valero *et al.*, 2012
RQ-00203078 (10 μM)	TRPM8 blockade	Oral squamous cell	Cell migration and invasion suppression	SOCE ↓, MMP-9 activity ↓	Okamoto *et al.*, 2012

TRPM7

Waixenicin A is an extract from *Sarcothelia edmondsoni* (syn. *Anthelia edmondsoni*), a soft coral from Hawaii, that inhibits TRPM7-mediated Mn^{2+} quenching of fura-2 fluorescence in a dose-dependent manner with an EC_{50} of 12 μM (Zierler *et al.*, 2011). Moreover, waixenicin A inhibits the TRPM7 whole-cell currents with an EC_{50} of 7 μM. The inhibitory effects of waixenicin A on TRPM7 are strongly dependent on $[Mg^{2+}]_i$, indicating that this compound enhances Mg^{2+} block of the channels or that Mg^{2+} enhances binding affinity of waixenicin A. Importantly, waixenicin A was without effect on TRPM6 channels, the closest homologues of TRPM7 channels. Then, Zierler *et al.* showed that waixenicin A is a promising candidate as a specific and potent blocker of TRPM7 in a wide range of diseases including cancers (Zierler *et al.*, 2011). Recently, Kim *et al.* showed that waixenicin A (from 30 to 50 μM) is a potent inhibitor of gastric and breast cancer proliferation in a TRPM7-dependent manner (Kim *et al.*, 2013). Indeed, waixenicin A was able to inhibit gastric cancer AGS cell and breast cancer MCF-7 cell proliferation as well as reducing the TRPM7 currents in these cell lines.

Moreover, ginsenoside Rd, one of the more active ginseng saponin components, has been shown to block TRPM7 channels and to induce cell death in gastric and breast cancer cells (Kim, 2013). Indeed, ginsenoside Rd decreased cell viability in MCF-7 and AGS in a concentration-dependent manner with an EC_{50} of 154.3 μM for MCF-7 cells and 131.2 μM for AGS cells, while it increased cell viability in normal HEK293 cells. Ginsenoside Rd-induced cell death was due to intrinsic apoptosis signalling via mitochondrial membrane depolarization. Moreover, ginsenoside Rd increased caspase-3 activity in both MCF-7 and AGS cancer cells. Importantly, ginsenoside Rd inhibited TRPM7 currents in MCF-7 and AGS, with median inhibitory concentrations of 178 μM and 170 μM, respectively.

TRPM8

TRPM8 is a promising target in prostate cancer (Zhang and Barritt, 2006). TRPM8 blockers such as 4-(3-chloropyridin-2-yl)-piperazine-1-carboxylic acid (4-tertbutylphenyl)-amide (BCTC), clotrimazole, and [L-arginyl]-[*N*-[2,4-dichlorophenethyl]glycyl]-*N*-(2,4-dichlorophenethyl) glycinamide (DD01050), as well as more specific blockers like *N*-(3-aminopropyl)-2-[(3-methylphenyl)methoxy]-*N*-(2-thienylmethyl)-benzamidehydrochloride (1:1) hyclate (AMTB) and 3-[7-trifluoromethyl-5-(2-trifluoromethylphenyl)-1H-benzimidazol-2-yl]-1-oxa-2-aza-spiro[4.5]dec-2-ene hydrochloride (JNJ41876666) have been used in different human prostate cancer cell lines (Valero *et al.*, 2011; 2012). BCTC, clotrimazole, AMTB and JNJ41876666 at a concentration of 10 μM all inhibited cell proliferation in all prostate cancer cell lines, but not in normal prostate cells. Moreover, RQ-00203078 (RQ), another TRPM8 antagonist, was used in HSC3 and HSC4 oral squamous cell carcinoma cell lines (Okamoto *et al.*, 2012). RQ at 10 μM completely abolished the menthol-induced TRPM8 whole-cell currents and SOCE in both cell lines. Moreover, RQ inhibited both menthol-induced and basal cell proliferation as well as menthol-induced and basal migration and invasion of cells. Finally, menthol-induced MMP-9 activity was also suppressed by RQ (Okamoto *et al.*, 2012).

TRPA1 targeting in cancer

TRPA1 is the only member of the TRPA group and is mainly expressed in sensory neurons as well as in hair and skin cells (Wu *et al.*, 2010). TRPA1 is considered to be a chemosensor and is implicated in pain-associated TRP channelopathy (Kremeyer *et al.*, 2010). TRPA1 is also expressed in the vasculature (Zholos, 2010; Earley, 2012; Fernandes *et al.*, 2012) and in the gut (Izzo *et al.*, 2012). Although TRPA1 has not been shown to be involved in cancer directly, it is implicated in the intestinal inflammation that is a well-known condition associated with colon cancer. Indeed, TRPA1 antagonism ameliorates experimental colitis (Engel *et al.*, 2011), and TRPA1 mRNA is up-regulated in inflamed gut (Izzo *et al.*, 2012). Moreover, blockade of the TRPA1 channel by its antagonist HC-030031 is used to prevent chemotherapy-induced peripheral neuropathy (CIPN) (Trevisan *et al.*, 2013). In contrast, inhaled activators of TRPA1 like allyl isothiocyanate (AITC) promote cell survival and proliferation in human small-cell lung cancer (Schaefer *et al.*, 2013). AITC (30 μM) increased $[Ca^{2+}]_i$ in a TRPA1-dependent manner and this effect was antagonized by camphor (2 mM) and by a more specific blocker of TRPA1, AP18 (10 μM). An increase in $[Ca^{2+}]_i$ induced by TRPA1 activation led to Ca^{2+}- and Src-dependent phosphorylation of ERK1/2 in human small-cell lung cancer cells, leading to cell survival and enhanced proliferation. Therefore, these results should be interpreted cautiously, because AITC may also have pharmacological effects that are independent of TRPA1 activation, as reported by Capasso *et al.* in mouse gastrointestinal tract (Capasso *et al.*, 2012).

Limitations of pharmacological approaches used to target TRP channels in cancer

In this review, we aimed to provide an update of recent knowledge regarding pharmacological strategies used to target TRP channels in cancer. Most of these studies employed channel regulators to study their role in cancer cell responses. While the use of such cancer cell models provides interesting conceptual information on cellular and molecular mechanisms that can drive the malignant progression, the development of new therapeutic strategies based on TRP channel blockers is still in its infancy. We and others have demonstrated that TRP channel expression is altered in malignant tissues in correlation with clinical parameters, allowing us to propose these proteins as potential biomarkers in cancer (Zhang and Barritt, 2006; Prevarskaya *et al.*, 2007; Ouadid-Ahidouch *et al.*, 2013). These findings support the relevance of studying TRP's role in cancer for development of new therapeutic strategies. However, these molecular targets are far from ready to be used in preclinical trials, and future

studies that aim to compare TRP channel expression with established biomarkers in cancer are urgently needed. To our knowledge, only the TRPM8 agonist D-3263-hydrochloride (Dendreon Corporation, Seattle, WA, USA), which increases cell death in TRPM8-expressing tumour cells, has been tested clinically. Indeed, preclinical data on 15 patients (phase 1 clinical trials) show disease stabilization in men with advanced prostate cancer. Moreover, the radiohalogen form of the TRPM8 agonist SW-12-18F could subsequently be used for radiotherapeutic treatments (Beck *et al*., 2007).

In parallel with these clinical studies, there is also an urgent need to develop more specific pharmacological agents, which should be potent in the nanomolar range, as all the blockers used in the studies presented here are only potent at best in the micromolar range.

New TRP channel targeting tools in development

It has now been widely established that TRP channels play a role in oncology. Nevertheless, there is still a lack of knowledge regarding the use of TRP channels as therapeutic targets. Several strategies have been used to specifically block TRP channels in cancer cells.

For example, pore-blocking antibodies targeting TRPC1 and TRPC3/6 have been used to inhibit proliferation in the human lung adenocarcinoma epithelial cell line A549 (Jiang *et al*., 2013). Moreover, pore-blocking antibodies used against TRPM3 (Naylor *et al*., 2008) are also effective on TRPC channels (Xu, 2011), suggesting a potential therapeutic use of these molecules in TRPC-expressing tumours. Another strategy was the use of dominant-negative forms of TRP that are expressed in cancer cells via infection with adenovirus. In fact, dominant-negative forms of TRPC6 have been used to induce cell cycle arrest in glioma and oesophageal cancer (Ding *et al*., 2010a; 2010b).

Besides their specific role in cancer, TRP channels are emerging targets that have attracted pharmaceutical interest, particularly TRPA1, TRPV1, TRPV2 and TRPM8. The side effects and limited efficacy of TRPV1 agonists have thus far prevented any compounds from progressing beyond phase II clinical trials, but resiniferatoxin, a natural analogue of capsaicin, has recently been proposed as an attractive alternative to capsaicin in treating pain (Kissin and Szallasi, 2011). As analgesic treatment with resiniferatoxin does not exhibit the side effects associated with capsaicin, the authors suggested further testing of this molecule in the case of cancer-associated pain. Nevertheless, the effect of resiniferatoxin on thermoregulation needs to be assessed and controlled. In addition, De Petrocellis *et al*. (2011) recently showed that phytocannabinoids, including all pure plant cannabinoids and all *Cannabis* botanical substances, can activate TRPV1, TRPV2 and TRPA1 and antagonize TRPM8 channels. Indeed, these four latter TRP channels could be considered as ionotropic cannabinoid receptors, reinforcing the potential of these TRP channels as possible therapeutic targets as well as the use of cannabinoids as pharmacological tools. Nevertheless, the modulation of native TRP by cannabinoids in cancer cells still needs further investigation.

Conclusion and perspectives

Recent findings have proposed that TRP channels represent promising targets in cancer management, as their expression and activity regulate specific stages of cancer development and progression. Use of TRP channels in cancer treatment modalities may turn out to be particularly important due to tumour heterogeneity and development of chemoresistance, with the focus being placed on personalized therapy in patients with known levels of channel expression. As such, the TRPV6 (soricidin derivatives) and TRPM7 (waixenicin and ginsenoside Rd) inhibitors may offer attractive therapeutic opportunities in future treatment of hormone-dependent and -independent cancers, respectively, and the TRPM8 agonist (D-3263-hydrochloride) may offer the same for the treatment of prostate cancer. This justifies further exploration of these molecules for validation in cancer therapy, particularly of chemoresistant tumours and for a combinational approach. In addition, they can be beneficial for controlling accompanying processes like angiogenesis. However, the characterization of their involvement in tumour angiogenesis is still preliminary (Munaron *et al*., 2013). In summary, due to lack of early diagnostic markers and effective therapeutics, cancer remains one of the leading causes of mortality and morbidity worldwide. Therefore, the emergence of new molecular tools is required to improve patient survival and quality of life. It is necessary to overcome limitations by developing specific and highly potent blockers targeting TRP channels to fulfil the potential of this developing and promising research area. Further studies are crucial for the development of future clinical tools with a focus on a personalized therapeutic approach in patients with altered TRP channel status.

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Conflict of interest

None.

References

Alexander S PH, Benson H E, Faccenda E, Pawson A J, Sharman J L, Catterall W A, Spedding M, Peters J A, Harmar A J and CGTP Collaborators (2013). The Concise Guide to PHARMACOLOGY 2013/14: Ion Channels. Br J Pharmacol 170: 1607–1651.

Amantini C, Mosca M, Nabissi M, Lucciarini R, Caprodossi S, Arcella A *et al*. (2007). Capsaicin-induced apoptosis of glioma cells is mediated by TRPV1 vanilloid receptor and requires p38 MAPK activation. J Neurochem 102: 977–990.

Amantini C, Ballarini P, Caprodossi S, Nabissi M, Morelli MB, Lucciarini R *et al*. (2009). Triggering of transient receptor potential

vanilloid type 1 (TRPV1) by capsaicin induces Fas/CD95-mediated apoptosis of urothelial cancer cells in an ATM-dependent manner. Carcinogenesis 30: 1320–1329.

Azad N, Perroy A, Gardner E, Imamura CK, Graves C, Sarosy GA *et al.* (2009). A phase I study of paclitaxel and continuous daily CAI in patients with refractory solid tumors. Cancer Biol Ther 8: 1800–1805.

Beck B, Bidaux G, Bavencoffe A, Lemonnier L, Thebault S, Shuba Y *et al.* (2007). Prospects for prostate cancer imaging and therapy using high-affinity TRPM8 activators. Cell Calcium 41: 285–294.

Berna-Erro A, Redondo PC, Rosado JA (2012). Store-operated Ca^{2+} entry. Adv Exp Med Biol 740: 349–382.

Bode AM, Dong Z (2011). The two faces of capsaicin. Cancer Res 71: 2809–2814.

Bomben VC, Sontheimer H (2010). Disruption of transient receptor potential canonical channel 1 causes incomplete cytokinesis and slows the growth of human malignant gliomas. Glia 58: 1145–1156.

Bomben VC, Sontheimer HW (2008). Inhibition of transient receptor potential canonical channels impairs cytokinesis in human malignant gliomas. Cell Prolif 41: 98–121.

Bomben VC, Turner KL, Barclay TT, Sontheimer H (2011). Transient receptor potential canonical channels are essential for chemotactic migration of human malignant gliomas. J Cell Physiol 226: 1879–1888.

Bon RS, Beech DJ (2013). In pursuit of small molecule chemistry for calcium-permeable non-selective TRPC channels – mirage or pot of gold? Br J Pharmacol 170: 459–474.

Bowen CV, DeBay D, Ewart HS, Gallant P, Gormley S, Ilenchuk TT *et al.* (2013). In vivo detection of human TRPV6-rich tumors with anti-cancer peptides derived from soricidin. Plos ONE 8: e58866.

Brederson JD, Kym PR, Szallasi A (2013). Targeting TRP channels for pain relief. Eur J Pharmacol 716: 61–76.

Cai R, Ding X, Zhou K, Shi Y, Ge R, Ren G *et al.* (2009). Blockade of TRPC6 channels induced G2/M phase arrest and suppressed growth in human gastric cancer cells. Int J Cancer 125: 2281–2287.

Capasso R, Aviello G, Romano B, Borrelli F, De Petrocellis L, Di Marzo V *et al.* (2012). Modulation of mouse gastrointestinal motility by allyl isothiocyanate, a constituent of cruciferous vegetables (Brassicaceae): evidence for TRPA1-independent effects. Br J Pharmacol 165: 1966–1977.

Caprodossi S, Amantini C, Nabissi M, Morelli MB, Farfariello V, Santoni M *et al.* (2011). Capsaicin promotes a more aggressive gene expression phenotype and invasiveness in null-TRPV1 urothelial cancer cells. Carcinogenesis 32: 686–694.

Chow J, Norng M, Zhang J, Chai J (2007). TRPV6 mediates capsaicin-induced apoptosis in gastric cancer cells – mechanisms behind a possible new 'hot' cancer treatment. Biochim Biophys Acta 1773: 565–576.

De Petrocellis L, Ligresti A, Moriello AS, Allara M, Bisogno T, Petrosino S *et al.* (2011). Effects of cannabinoids and cannabinoid-enriched *Cannabis* extracts on TRP channels and endocannabinoid metabolic enzymes. Br J Pharmacol 163: 1479–1494.

Ding X, He Z, Shi Y, Wang Q, Wang Y (2010a). Targeting TRPC6 channels in oesophageal carcinoma growth. Expert Opin Ther Targets 14: 513–527.

Ding X, He Z, Zhou K, Cheng J, Yao H, Lu D *et al.* (2010b). Essential role of TRPC6 channels in G2/M phase transition and development of human glioma. J Natl Cancer Inst 102: 1052–1068.

Duncan LM, Deeds J, Hunter J, Shao J, Holmgren LM, Woolf EA *et al.* (1998). Down-regulation of the novel gene melastatin correlates with potential for melanoma metastasis. Cancer Res 58: 1515–1520.

Earley S (2012). TRPA1 channels in the vasculature. Br J Pharmacol 167: 13–22.

Engel MA, Leffler A, Niedermirtl F, Babes A, Zimmermann K, Filipovic MR *et al.* (2011). TRPA1 and substance P mediate colitis in mice. Gastroenterology 141: 1346–1358.

Fernandes ES, Fernandes MA, Keeble JE (2012). The functions of TRPA1 and TRPV1: moving away from sensory nerves. Br J Pharmacol 166: 510–521.

Gkika D, Prevarskaya N (2011). TRP channels in prostate cancer: the good, the bad and the ugly? Asian J Androl 13: 673–676.

Guilbert A, Gautier M, Dhennin-Duthille I, Haren N, Sevestre H, Ouadid-Ahidouch H (2009). Evidence that TRPM7 is required for breast cancer cell proliferation. Am J Physiol Cell Physiol 297: C493–C502.

Guilbert A, Gautier M, Dhennin-Duthille I, Rybarczyk P, Sahni J, Sevestre H *et al.* (2013). Transient receptor potential melastatin 7 is involved in oestrogen receptor-negative metastatic breast cancer cells migration through its kinase domain. Eur J Cancer 49: 3694–3707.

Harper JL, Camerini-Otero CS, Li AH, Kim SA, Jacobson KA, Daly JW (2003). Dihydropyridines as inhibitors of capacitative calcium entry in leukemic HL-60 cells. Biochem Pharmacol 65: 329–338.

He B, Liu F, Ruan J, Li A, Chen J, Li R *et al.* (2012). Silencing TRPC1 expression inhibits invasion of CNE2 nasopharyngeal tumor cells. Oncol Rep 27: 1548–1554.

Hoenderop JG, Nilius B, Bindels RJ (2005). Calcium absorption across epithelia. Physiol Rev 85: 373–422.

Hu HZ, Gu Q, Wang C, Colton CK, Tang J, Kinoshita-Kawada M *et al.* (2004). 2-aminoethoxydiphenyl borate is a common activator of TRPV1, TRPV2, and TRPV3. J Biol Chem 279: 35741–35748.

Hwang JA, Hwang MK, Jang Y, Lee EJ, Kim JE, Oh MH *et al.* (2013). 20-*O*-beta-D-glucopyranosyl-20(*S*)-protopanaxadiol, a metabolite of ginseng, inhibits colon cancer growth by targeting TRPC channel-mediated calcium influx. J Nutr Biochem 24: 1096–1104.

Izzo AA, Capasso R, Aviello G, Borrelli F, Romano B, Piscitelli F *et al.* (2012). Inhibitory effect of cannabichromene, a major non-psychotropic cannabinoid extracted from *Cannabis sativa*, on inflammation-induced hypermotility in mice. Br J Pharmacol 166: 1444–1460.

Jiang HN, Zeng B, Zhang Y, Daskoulidou N, Fan H, Qu JM *et al.* (2013). Involvement of TRPC channels in lung cancer cell differentiation and the correlation analysis in human non-small cell lung cancer. PLoS ONE 8: e67637.

Johnson EA, Marks RS, Mandrekar SJ, Hillman SL, Hauge MD, Bauman MD *et al.* (2008). Phase III randomized, double-blind study of maintenance CAI or placebo in patients with advanced non-small cell lung cancer (NSCLC) after completion of initial therapy (NCCTG 97-24-51). Lung Cancer 60: 200–207.

Kim BJ (2013). Involvement of melastatin type transient receptor potential 7 channels in ginsenoside Rd-induced apoptosis in gastric and breast cancer cells. J Ginseng Res 37: 201–209.

Kim BJ, Nam JH, Kwon YK, So I, Kim SJ (2013). The role of waixenicin A as transient receptor potential melastatin 7 blocker. Basic Clin Pharmacol Toxicol 112: 83–89.

Kissin I, Szallasi A (2011). Therapeutic targeting of TRPV1 by resiniferatoxin, from preclinical studies to clinical trials. Curr Top Med Chem 11: 2159–2170.

Kohn EC, Figg WD, Sarosy GA, Bauer KS, Davis PA, Soltis MJ *et al.* (1997). Phase I trial of micronized formulation carboxyamidotriazole in patients with refractory solid tumors: pharmacokinetics, clinical outcome, and comparison of formulations. J Clin Oncol 15: 1985–1993.

Kremeyer B, Lopera F, Cox JJ, Momin A, Rugiero F, Marsh S *et al.* (2010). A gain-of-function mutation in TRPA1 causes familial episodic pain syndrome. Neuron 66: 671–680.

Lehen'kyi V, Raphael M, Prevarskaya N (2012). The role of the TRPV6 channel in cancer. J Physiol 590 (Pt 6): 1369–1376.

Maruyama T, Kanaji T, Nakade S, Kanno T, Mikoshiba K (1997). 2APB, 2-aminoethoxydiphenyl borate, a membrane-penetrable modulator of Ins(1,4,5)P3-induced Ca^{2+} release. J Biochem 122: 498–505.

Middelbeek J, Kuipers AJ, Henneman L, Visser D, Eidhof I, van Horssen R *et al.* (2012). TRPM7 is required for breast tumor cell metastasis. Cancer Res 72: 4250–4261.

Mikkelsen T, Lush R, Grossman SA, Carson KA, Fisher JD, Alavi JB *et al.* (2007). Phase II clinical and pharmacologic study of radiation therapy and carboxyamido-triazole (CAI) in adults with newly diagnosed glioblastoma multiforme. Invest New Drugs 25: 259–263.

Monet M, Gkika D, Lehen'kyi V, Pourtier A, Vanden Abeele F, Bidaux G *et al.* (2009). Lysophospholipids stimulate prostate cancer cell migration via TRPV2 channel activation. Biochim Biophys Acta 1793: 528–539.

Moran MM, McAlexander MA, Biro T, Szallasi A (2011). Transient receptor potential channels as therapeutic targets. Nat Rev Drug Discov 10: 601–620.

Morelli MB, Nabissi M, Amantini C, Farfariello V, Ricci-Vitiani L, Di Martino S *et al.* (2012). The transient receptor potential vanilloid-2 cation channel impairs glioblastoma stem-like cell proliferation and promotes differentiation. Int J Cancer 131: E1067–E1077.

Munaron L, Genova T, Avanzato D, Antoniotti S, Fiorio Pla A (2013). Targeting calcium channels to block tumor vascularization. Recent Pat Anticancer Drug Discov 8: 27–37.

Nabissi M, Morelli MB, Santoni M, Santoni G (2013). Triggering of the TRPV2 channel by cannabidiol sensitizes glioblastoma cells to cytotoxic chemotherapeutic agents. Carcinogenesis 34: 48–57.

Naylor J, Milligan CJ, Zeng F, Jones C, Beech DJ (2008). Production of a specific extracellular inhibitor of TRPM3 channels. Br J Pharmacol 155: 567–573.

Nilius B, Owsianik G, Voets T, Peters JA (2007). Transient receptor potential cation channels in disease. Physiol Rev 87: 165–217.

Okamoto Y, Ohkubo T, Ikebe T, Yamazaki J (2012). Blockade of TRPM8 activity reduces the invasion potential of oral squamous carcinoma cell lines. Int J Oncol 40: 1431–1440.

Ouadid-Ahidouch H, Dhennin-Duthille I, Gautier M, Sevestre H, Ahidouch A (2013). TRP channels: diagnostic markers and therapeutic targets for breast cancer? Trends Mol Med 19: 117–124.

Oulidi A, Bokhobza A, Gkika D, Vanden Abeele F, Lehen'kyi V, Ouafik L *et al.* (2013). TRPV2 mediates adrenomedullin stimulation of prostate and urothelial cancer cell adhesion, migration and invasion. PLoS ONE 8: e64885.

Preti D, Szallasi A, Patacchini R (2012). TRP channels as therapeutic targets in airway disorders: a patent review. Expert Opin Ther Pat 22: 663–695.

Prevarskaya N, Zhang L, Barritt G (2007). TRP channels in cancer. Biochim Biophys Acta 1772: 937–946.

Prevarskaya N, Skryma R, Shuba Y (2011). Calcium in tumour metastasis: new roles for known actors. Nat Rev Cancer 11: 609–618.

Ramer R, Hinz B (2008). Inhibition of cancer cell invasion by cannabinoids via increased expression of tissue inhibitor of matrix metalloproteinases-1. J Natl Cancer Inst 100: 59–69.

Ramer R, Merkord J, Rohde H, Hinz B (2010). Cannabidiol inhibits cancer cell invasion via upregulation of tissue inhibitor of matrix metalloproteinases-1. Biochem Pharmacol 79: 955–966.

Romanovsky AA, Almeida MC, Garami A, Steiner AA, Norman MH, Morrison SF *et al.* (2009). The transient receptor potential vanilloid-1 channel in thermoregulation: a thermosensor it is not. Pharmacol Rev 61: 228–261.

Rybarczyk P, Gautier M, Hague F, Dhennin-Duthille I, Chatelain D, Kerr-Conte J *et al.* (2012). Transient receptor potential melastatin-related 7 channel is overexpressed in human pancreatic ductal adenocarcinomas and regulates human pancreatic cancer cell migration. Int J Cancer 131: E851–E861.

Sanchez AM, Sanchez MG, Malagarie-Cazenave S, Olea N, Diaz-Laviada I (2006). Induction of apoptosis in prostate tumor PC-3 cells and inhibition of xenograft prostate tumor growth by the vanilloid capsaicin. Apoptosis 11: 89–99.

Santoni G, Farfariello V (2011). TRP channels and cancer: new targets for diagnosis and chemotherapy. Endocr Metab Immune Disord Drug Targets 11: 54–67.

Schaefer EA, Stohr S, Meister M, Aigner A, Gudermann T, Buech TR (2013). Stimulation of the chemosensory TRPA1 cation channel by volatile toxic substances promotes cell survival of small cell lung cancer cells. Biochem Pharmacol 85: 426–438.

Shapovalov G, Lehen'kyi V, Skryma R, Prevarskaya N (2011). TRP channels in cell survival and cell death in normal and transformed cells. Cell Calcium 50: 295–302.

Shi Y, Ding X, He ZH, Zhou KC, Wang Q, Wang YZ (2009). Critical role of TRPC6 channels in G2 phase transition and the development of human oesophageal cancer. Gut 58: 1443–1450.

Singh A, Hildebrand ME, Garcia E, Snutch TP (2010). The transient receptor potential channel antagonist SKF96365 is a potent blocker of low-voltage-activated T-type calcium channels. Br J Pharmacol 160: 1464–1475.

Stadler WM, Rosner G, Small E, Hollis D, Rini B, Zaentz SD *et al.* (2005). Successful implementation of the randomized discontinuation trial design: an application to the study of the putative antiangiogenic agent carboxyaminoimidazole in renal cell carcinoma – CALGB 69901. J Clin Oncol 23: 3726–3732.

Stock K, Kumar J, Synowitz M, Petrosino S, Imperatore R, Smith ES *et al.* (2012). Neural precursor cells induce cell death of high-grade astrocytomas through stimulation of TRPV1. Nat Med 18: 1232–1238.

Sung B, Prasad S, Ravindran J, Yadav VR, Aggarwal BB (2012). Capsazepine, a TRPV1 antagonist, sensitizes colorectal cancer cells to apoptosis by TRAIL through ROS-JNK-CHOP-mediated upregulation of death receptors. Free Radic Biol Med 53: 1977–1987.

Trevisan G, Materazzi S, Fusi C, Altomare A, Aldini G, Lodovici M *et al.* (2013). Novel therapeutic strategy to prevent chemotherapy-induced persistent sensory neuropathy by TRPA1 blockade. Cancer Res 73: 3120–3131.

Valero M, Morenilla-Palao C, Belmonte C, Viana F (2011). Pharmacological and functional properties of TRPM8 channels in prostate tumor cells. Pflugers Arch 461: 99–114.

Valero ML, Mello de Queiroz F, Stuhmer W, Viana F, Pardo LA (2012). TRPM8 ion channels differentially modulate proliferation and cell cycle distribution of normal and cancer prostate cells. PLoS ONE 7: e51825.

Vay L, Gu C, McNaughton PA (2012). The thermo-TRP ion channel family: properties and therapeutic implications. Br J Pharmacol 165: 787–801.

Venkatachalam K, Montell C (2007). TRP channels. Annu Rev Biochem 76: 387–417.

Vennekens R, Owsianik G, Nilius B (2008). Vanilloid transient receptor potential cation channels: an overview. Curr Pharm Des 14: 18–31.

Waning J, Vriens J, Owsianik G, Stuwe L, Mally S, Fabian A *et al*. (2007). A novel function of capsaicin-sensitive TRPV1 channels: involvement in cell migration. Cell Calcium 42: 17–25.

Wu LJ, Sweet TB, Clapham DE (2010). International Union of Basic and Clinical Pharmacology. LXXVI. Current progress in the mammalian TRP ion channel family. Pharmacol Rev 62: 381–404.

Xu SZ (2011). Assessing TRPC channel function using pore-blocking antibodies. 2012/05/18 edn. Zhu RTX (ed.). TRP Channels. CRC Press: Boca Raton, FL, pp. 149–166.

Zhang L, Barritt GJ (2006). TRPM8 in prostate cancer cells: a potential diagnostic and prognostic marker with a secretory function? Endocr Relat Cancer 13: 27–38.

Zholos A (2010). Pharmacology of transient receptor potential melastatin channels in the vasculature. Br J Pharmacol 159: 1559–1571.

Zierler S, Yao G, Zhang Z, Kuo WC, Porzgen P, Penner R *et al*. (2011). Waixenicin A inhibits cell proliferation through magnesium-dependent block of transient receptor potential melastatin 7 (TRPM7) channels. J Biol Chem 286: 39328–39335.