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REVIEW

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Ca_V channels and cancer: canonical functions indicate benefits of repurposed drugs as cancer therapeutics

Paul J. Buchanan[1,2] · Karen D. McCloskey[1]

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Abstract The importance of ion channels in the hallmarks of many cancers is increasingly recognised. This article reviews current knowledge of the expression of members of the voltage-gated calcium channel family (Ca_V) in cancer at the gene and protein level and discusses their potential functional roles. The ten members of the Ca_V channel family are classified according to expression of their pore-forming α-subunit; moreover, co-expression of accessory α2δ, β and γ confers a spectrum of biophysical characteristics including voltage dependence of activation and inactivation, current amplitude and activation/inactivation kinetics. Ca_V channels have traditionally been studied in excitable cells including neurones, smooth muscle, skeletal muscle and cardiac cells, and drugs targeting the channels are used in the treatment of hypertension and epilepsy. There is emerging evidence that several Ca_V channels are differentially expressed in cancer cells compared to their normal counterparts. Interestingly, a number of Ca_V channels also have non-canonical functions and are involved in transcriptional regulation of the expression of other proteins including potassium channels. Pharmacological studies show that Ca_V canonical function contributes to the fundamental biology of proliferation, cell-cycle progression and apoptosis. This raises the intriguing possibility that calcium channel blockers, approved for the treatment of other conditions, could be repurposed to treat particular cancers. Further research will reveal the full extent of both the canonical and non-canonical functions of Ca_V channels in cancer and whether calcium channel blockers are beneficial in cancer treatment.

Keywords Cancer · Calcium channels · Repurposed drugs

Introduction

Calcium signalling is an important physiological property of cells given the essential roles of calcium ions (Ca^{2+}) in processes such as contraction, motility, apoptosis, transmitter release, exocytosis and endocytosis. Cells have many mechanisms for the precise regulation of intracellular Ca^{2+} concentration including ion channels [TRPs and voltage-gated calcium channels (VGCCs)], transporters and pumps on the plasma membrane and intracellular membranes, e.g., Na^+/Ca^{2+} exchanger. VGCCs have been widely studied in the context of excitable cells in cardiovascular physiology, neuromuscular physiology and neuroscience, and their inhibition by several classes of calcium channel blockers (CCBs) is important in the treatment of hypertension and epilepsy.

Interestingly, VGCCs are functionally expressed in non-excitable cells including immune cells (Vig and Kinet 2009; Davenport et al. 2015) and a number of epithelial cancer cell types (Prevarskaya et al. 2010, 2014; Lastraioli et al. 2015; Wang et al. 2015). These cells also express TRP channels and it was considered that calcium signalling operated mainly via store-operated calcium channels, now defined molecularly as interactions between Orai channel

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✉ Karen D. McCloskey
k.mccloskey@qub.ac.uk

[1] Centre for Cancer Research and Cell Biology, School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, 97 Lisburn Road, Belfast, Northern Ireland BT9 7AE, UK

[2] Present Address: National Institute of Cellular Biotechnology, School of Nursing and Human Science, Dublin City University, Glasnevin, Dublin 9, Ireland

proteins on the plasma membrane and STIM proteins on the endoplasmic reticulum (Soboloff et al. 2012; Hogan et al. 2010). It is now known that a diverse array of VGCCs is functionally active in non-excitable cells and contribute to Ca^{2+}-dependent signalling processes. In cancer cells, VGCCs are involved in several of the cancer hallmarks, originally described by Hanahan and Weinberg (2000) as sustaining proliferative signalling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis and more recently updated (Hanahan and Weinberg 2011) to include the enabling hallmarks of reprogramming energy metabolism and evading immune destruction.

Ca_V ion channel family

The VGCC family comprises ten members, based on expression of a specific pore-forming α_1-subunit of 190–250 kDa containing the voltage sensor and binding sites for modulators and drugs and divided into three phylogenetic subfamilies: Ca_V1, Ca_V2 and Ca_V3 (Ertel et al. 2000; Catterall 2011; Catterall et al. 2005; Alexander et al. 2015). In the approved nomenclature 'Ca' represents Ca^{2+} as the main permeating ion, 'V' indicating the physiological modulator, voltage and the number of the subfamily followed by its α1 subunit. The Ca_V1 subfamily includes $Ca_V1.1$ (α_{1S}), $Ca_V1.2$ (α_{1C}), $Ca_V1.3$ (α_{1D}) and $Ca_V1.4$ (α_{1F}), known as L-type channels, describing 'long-lasting currents', which are typically high voltage-activated and dihydropyridine-sensitive. $Ca_V2.1$, $Ca_V2.2$ and $Ca_V2.3$, are high voltage-activated and dihydropyridine-insensitive channels which contain α_{1A}, α_{1B} and α_{1E} subunits, respectively, mediating P/Q-type, N-type and R-type Ca^{2+} currents. Ca_V3 channels $Ca_V3.1$ (α_{1G}), $Ca_V3.2$ (α_{1H}) and $Ca_V3.3$ (α_{1I}) are low voltage-activated, dihydropyridine-sensitive, T-type or 'transient currents' indicating their kinetics of activation and inactivation (Catterall et al. 2005). L-type and T-type Ca_V families are expressed in many cell types while N, P/Q, and R-type channels are predominantly expressed in neurons. Alternative splicing of the pore-forming α subunits confers unique pharmacological and electrophysiological properties to VGCC representing remarkable plasticity and molecular diversity (Hofmann et al. 1994; Tan et al. 2011; Fan et al. 2005; Gray et al. 2007; Singh et al. 2008; Huang et al. 2013). It has been estimated that there are over 1000 theoretical splice isoforms from a single α1 subunit, based on known splice sites (Fox et al. 2008; Emerick et al. 2006; Gray et al. 2007).

Accessory subunits

Activity of Ca_V channels is modulated by co-expression of a number of accessory subunits, α2δ, β and γ, which themselves have several members, α2δ1, α2δ2, α2δ3, α2δ4, β, β2, β3, β4 and γ. The emerging role of these subunits has recently been reviewed by Hofmann et al. (2015) and includes essential physiological processes of channel trafficking and stability in the plasma membrane in addition to regulation of channel activity.

Four mammalian genes have been identified for the α2δ1, α2δ2, α2δ3 and α2δ4 subunits, which are CACNA2D1, CACNA2D2, CACNA2D3 and CACNA2D4 respectively (Klugbauer et al. 2003; Davies et al. 2007). These subunits act to increase current amplitude through $Ca_v\alpha_1$:$Ca_v\beta$ complexes, modify channel gating, induce a hyperpolarising shift in the voltage dependence of inactivation and modulate membrane stability in addition to being the binding sites for the anti-epileptic drugs gabapentin and pregabalin (Alexander et al. 2015).

$Ca_V\beta$1-4 subunits are cytosolic proteins that act to regulate current density by controlling the amount of α_1 subunit expressed at the cell membrane. In addition, β subunits regulate channel activation/inactivation kinetics and shift the voltage dependence of activation in the hyperpolarised direction. The $Ca_V\gamma$ subunits, of which eight have been identified, are structurally similar to the skeletal muscle $Ca_V\gamma1$ subunit but γ2–8 are known to be transmembrane proteins involved in regulation of trafficking and gating of AMPA receptors and may not be linked with Ca_V channel activity (Hofmann et al. 2015).

Electrophysiological properties

The Ca_V subfamilies have distinctive electrophysiological properties reflecting their molecular composition (see Catterall et al. 2005 for a comprehensive review). L-type Ca_V1 currents typically activate positively to −40 mV, peak at 0 mV in physiological solutions and show voltage dependence of activation and inactivation. These long-lasting currents show slow calcium-dependent inactivation, which is absent when Ba^{2+} is the predominant charge carrier. Neuronal N-type $Ca_V2.2$ channels have an intermediate voltage dependence and rate of inactivation that is faster than L-type and slower than T-type channels (Nowycky et al. 1985; Fox et al. 2008). T-type Ca_V3 channels normally activate at more negative potentials than L-type, around −60 mV, peak at −20 mV and have faster kinetics of activation and inactivation.

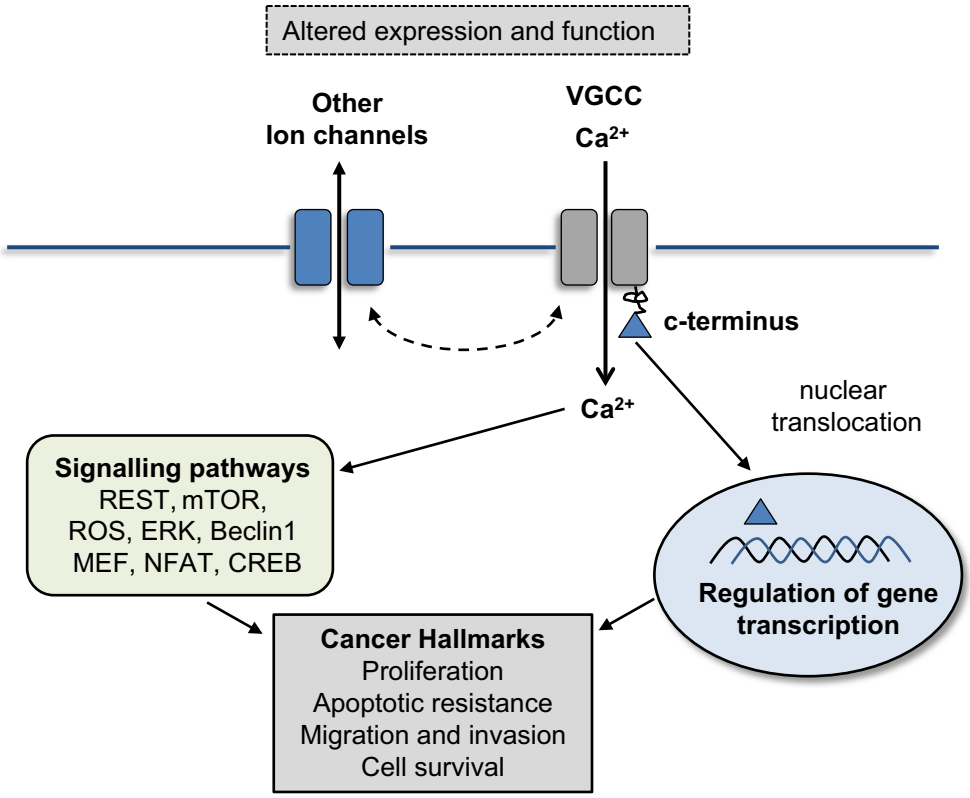

Fig. 1 Schematic of potential mechanisms of Ca_V channels in cancer. In many cancers, expression and function of Ca_V channels is altered. There is compelling evidence that altered Ca_V expression and function contributes to several cancer hallmarks including proliferation, apoptotic resistance, migration, invasion and enhanced cell survival. These can arise through Ca^{2+}-dependent signalling pathways via influx of Ca^{2+} through the Ca_V membrane channels. In addition, non-canonical signalling occurs, particularly in $Ca_V1.2$ and $Ca_V1.3$ where proteolytic cleavage of the c terminus produces a fragment that translocates to the nucleus and regulates the transcription of genes involved in processes of tumour development and progression

Pharmacology

Ca_V1 and Ca_V3 channels are sensitive to the dihydropyridine class of CCBs, which includes nifedipine, nimodipine, nisoldipine, felodipine and isradipine and can be activated by dihydropyridines such as Bay K8644. These drugs, rather than physically blocking the pore, act allosterically to shift the channel toward the open or closed state (Catterall et al. 2005). Within the Ca_V1 and Ca_V3 subfamilies, there is some differential sensitivity where $Ca_V1.2$ is more sensitive to nifedipine than $Ca_V1.3$ which is incompletely inhibited; moreover, Ca_V3 channels are relatively less sensitive (Stengel et al. 1998). Phenylalkylamines, e.g., verapamil, are intracellular pore blockers, which are thought to enter the pore from the cytoplasmic side of the channel and cause occlusion (Catterall et al. 2005). Ca_V1 channels are also sensitive to the CCB family of benzothiazepines such as diltiazem whereas Ca_V2 and Ca_V3 are not affected.

$Ca_V2.2$ N-type currents are insensitive to dihydropyridines but can be blocked by the cone snail peptide ω-conotoxin GVIA and other related peptide toxins (Tsien et al. 1988; Olivera et al. 1994). $Ca_V2.1$ P/Q currents have high sensitivity to the spider toxin ω-agatoxin IVA (Mintz et al. 1992) whereas Ca_V 2.3 R-type currents exhibit resistance to dihydropyridines but are sensitive to the tarantula-derived peptide SNX-482 (Newcomb et al. 1998).

Modulators of Ca_V3 channels include Ni^{2+} ions and the small molecule inhibitor mibefradil, which is widely used (Martin et al. 2000; Lee et al. 1999). $Ca_V3.1$ channels are blocked with kurtoxin, a peptide isolated from scorpion venom (Chuang et al.1998), although this is not selective as it also targets sodium channels. The diuretic amiloride blocks all Ca_V3 families with varying affinities but has other targets including sodium channels (Tang et al. 1988; Lopez-Charcas et al. 2012; Zamponi et al. 2015).

Expression of Ca_V in cancer

There is now compelling evidence that Ca_V channels are expressed in many cancers at the gene and protein level. Publically available data sets such as ONCOMINE are an

excellent resource for investigation of ion channel expression from transcriptomic analyses across many studies. Wang et al. (2015) recently published a comprehensive meta-analysis of public microarray data sets reporting VGCC gene signatures from cancer patient samples demonstrating remarkable expression of Ca_V genes. A significant number of research groups have selected particular ion channels and characterised their functional expression with patch-clamp electrophysiology in primary cells derived from tumours or established cell lines.

$Ca_V1.1$

The $Ca_V1.1$ gene (CACNA1S) is reportedly overexpressed in cancer compared with normal tissues in acute myeloid leukaemia, brain desmoplastic medulloblastoma and neuroectodermal tumours (Wang et al. 2015). Furthermore, this study revealed that CACNA1A was among the top 5 % upregulated genes in the leukaemia and brain cancer data sets. To date, the functional expression of $Ca_V1.1$ in these cancers has not been investigated and this is potentially an exciting area of research.

$Ca_V1.2$

Overexpression of $Ca_V1.2$ (gene CACNA1C) occurs in many cancers including colorectal, gastric, pancreatic, sarcoma, leukaemia, brain, breast, uterus, skin and prostate (Wang et al. 2015). This together with the finding that CACNA1C was in the top 10 % of upregulated genes may indicate involvement of $Ca_V1.2$ in common molecular mechanisms of definitive carcinogenic events. In human breast cancer MCF7 cells, increased $Ca_V1.2$ expression and reduced expression of the calcium binding protein, regucalcin, was induced by 5α-dihydrotestosterone (DHT), leading to reduced cell viability (Marques et al. 2015). Increased gene expression of CACNA1C has been reported for oesophageal squamous cell carcinoma, correlated with differentiation (Shi et al. 2015); however, there is no available information on protein expression or ion channel function. Similarly, a study of gene expression data from high-grade serous ovarian cancer patients showed 11 copy number amplified genes including CACNA1C (Davis et al. 2015). Wang et al. (2000) reported protein expression of CACNA1C, $Ca_V1.2α$, in human colon cancer tissue samples and in Caco-2 and T84 cells. Interestingly, Western blotting and immunofluorescence revealed that $Ca_V1.2α$ was increased in non-confluent colonic cancer cells leading the authors to conclude that the functional expression of $Ca_V1.2α$ was linked with proliferation.

Calcium mobilisation via L-type calcium channels such as $Ca_v1.2$ impacts other signalling mechanisms including activation of large-conductance, calcium-activated potassium channels, BK (Prakriya and Lingle 1999; Berkefeld et al. 2006). This is potentially important as BK channels are known to be involved in cancer biology (see the detailed review by Pardo and Stuhmer 2014) and $Ca_V1.2$ therefore may indirectly drive cancer hallmarks by regulating BK channel activity.

$Ca_V1.3$

A study of the effects of nifedipine on endometrial carcinoma Hec-1A cells showed decreased proliferation and migration in addition to induction of autophagy via Beclin1 and mTOR pathways (Bao et al. 2012). The same group also reported expression of CACNA1D and CACNA1G in these cells and found that proliferation and migration were sensitive to mibefradil or nifedipine (Bao et al. 2012). Consistent with this, $Ca_V1.3$ protein expression is reportedly higher in atypical hyperplasia and endometrial carcinoma tissues compared with benign endometrial tissue (Hao et al. 2015). Interestingly, $Ca_V1.3$ expression was enhanced by 17β-estradiol and its knockdown reduced the 17β-estradiol-mediated stimulation of Ca^{2+} influx, proliferation and migration in endometrial cancer Ishikawa cells (Hao et al. 2015). Together, this work indicates the importance of Ca^{2+} influx via $Ca_V1.3$ channels in endometrial carcinoma biology and further work should reveal the VGCC-related mechanisms underpinning this disease.

Electrophysiological patch-clamp experiments of MCF-7 breast cancer have demonstrated inward Ca^{2+} currents with the properties of both L and T-type Ca_V channels (Squecco et al. 2015). Pharmacological modulation with nifedipine or Ni^{2+} decreased cell proliferation confirming that Ca_V channels have physiological roles in breast cancer biology. Given that CACNA1C is known to be overexpressed in breast cancer tissue (Wang et al. 2015), it seems that $Ca_V1.2$ and one or more of the Ca_V3 subfamily are involved in the regulation of Ca^{2+} signalling in these cells.

$Ca_V1.3$ is co-expressed with other Ca_V channels and their subunits in a number of cancers including neuronal neuroblastoma cells (Park et al. 2010; Chiou 2006; Grassi et al. 2004; Kito et al. 1999; Neelands et al. 2000). Sousa et al. (2013) detected transcripts for $Ca_V1.3$, $Ca_V2.2$, $Ca_V3.1$ and a number of accessory subunits in SH-SY5Y human neuroblastoma cells. Depolarisation-induced Ca^{2+} events were nifedipine-sensitive and were also sensitive to the $Ca_V2.2$ inhibitors ω-conotoxins CVID, GVIA and MVIIA demonstrating functional activity of the channels at the plasma membrane. Currents with the biophysical and pharmacological properties of L, N- and P/Q and R type Ca_V channels have been recorded in neuroblastoma cells (Neelands et al. 2000) showing that these cells functionally express a number of distinct channels that regulate Ca^{2+} signalling. The role of this panel of Ca_V channels in cancer

Table 1 Summary of tumour expression of voltage-gated calcium channel (Ca_V) members and their associated functions to date

Ca_V member	Tumour expression	Function
$Ca_V1.1$	Brain, leukaemia	Not defined
$Ca_V1.2$	Brain, breast, colorectal, gastric, leukaemia, oesophageal, pancreatic, prostate, sarcoma, skin and uterine	Cell viability, proliferation, differentiation
$Ca_V1.3$	Breast, neuroblastoma, prostate, uterine	Proliferation, migration
$Ca_V1.4$	Testes	Not defined
$Ca_V2.1$	Cervical, leukaemia, ovarian, brain, uterine, ovarian, lung	Growth progression
$Ca_V2.2$	Breast, neuroblastoma, prostate	Not defined
$Ca_V2.3$	Kidney, oesophageal, ovarian, pancreatic and uterine	Non-canonical progression, proliferation
$Ca_V3.1$	Lung, pancreatic, neuroblastoma	Apoptotic resistance, autophagy, proliferation, cell cycle
$Ca_V3.2$	Breast, leukaemia, glioblastoma, prostate	Apoptotic resistance, differentiation, proliferation, survival
$Ca_V3.3$	Breast, colon, oesophageal, prostate, sarcoma	Proliferation

hallmarks of neuroblastoma has not yet been determined and represents a promising area of research.

There is a compelling body of evidence that $Ca_V1.3$ (CACNA1D) is overexpressed in prostate cancer at the gene and protein levels (Wang et al. 2015). Sun et al. (2006) reported that LNCaP prostate cancer cells displayed Ca^{2+} transients on stimulation with 5α-DHT and that these could be inhibited by the L-type channel inhibitors nifedipine,

diltiazem or verapamil. A study of CACNA1D in prostate cancer in the ONCOMINE database (Chen et al. 2014a) revealed its significant overexpression in cancer tissues compared with normal prostate, consistent with findings of CACNAID mRNA and $Ca_V1.3$ protein expression in prostate cancer cell lines. Interestingly the tumour microarray data showed that CACNA1D gene expression was higher in tumours with TMPRSS2-ERG fusion, which is in agreement with an epigenomic profiling study of prostate cancer tumours where CACNA1D was in the top-ranked differentially methylated genes in tissues with the TMPRSS2-ERG fusion (Geybels et al. 2015). Furthermore, other studies have reported CACNA1D to be in the top 10–20 genes most significantly correlated with ERG overexpression in patient tissues (Setlur et al. 2008; Jhavar et al. 2009; Boormans et al. 2013). In an evaluation of a panel of biomarkers to predict the aggressive prostate cancer phenotype, CACNA1D was correlated with Gleason score and biochemical recurrence (Zhu et al. 2015). Interestingly, high expression of CACNA1D was found to be an early event in active surveillance biopsies but in tumours with Gleason scores 4 + 3 or 8, CACNA1D was found to be lower. The significance of this finding needs to be investigated in other cohorts and experimental cell models.

Expression of $Ca_V1.3$ in normal prostate cells is apparently very low, yet there is early evidence that these cells utilise Ca^{2+}-signalling pathways, perhaps via other Ca_V channels. Connor et al. (1988) reported the dependence of hormonally induced prostate cell death on Ca^{2+}-influx pathways; moreover, Martikainen and Isaacs (1990) found that Ca^{2+}-dependent processes underpinned apoptosis induced by androgen removal.

It is interesting to note that the expression and function of $Ca_V1.2$ and $Ca_V1.3$ (discussed above) can be regulated by oestrogen and testosterone. This observation correlates with the high incidence of altered expression of these channels in the female and male reproductive systems (Table 1). Hormonal regulation of Ca_V1 channels and the impact of this on their role in the development of particular cancers is a promising area for further investigation.

$Ca_V1.4$

There is limited information on the expression of $Ca_V1.4$ (CACNA1F) in cancer. Mutations in CACNA1F cause the condition of incomplete congenital stationary night blindness (Striessnig et al. 2010). Wang et al. (2015) reported overexpression of CACAN1F in testicular teratoma in their meta-analysis of publically available TMA; functional expression of the $Ca_V1.4$ protein in this or other tumours has not yet been reported.

$Ca_V2.1$

P/Q type channels are overexpressed in a number of cancer types including leukaemia, ovarian carcinoma, sarcoma, brain cancers, uterine corpus leiomyoma, ovarian cancer, lung cancers and cervical cancer (Wang et al. 2015). There is emerging evidence that these channels functionally contribute to cancer biology. Around 50–60 % of patients with Lambert-Eaton syndrome, an autoimmune disease characterised by production of autoimmune P/Q type antibodies (Titulaer et al. 2011a), go on to develop small-cell lung cancer. This neuroendocrine cancer is known to contain functional VGCCs (Titulaer et al. 2011b) and patients with small-cell lung cancer with low levels of P/Q antibodies had poor survival compared to those of Lambert-Eaton syndrome with high levels of the antibody, suggesting that increased function of $Ca_V2.1$ P/Q channels may drive progression of the cancer (Roberts et al. 1985). This work also highlights the potential benefits of testing patients for the presence of these and other auto-immune antibodies in cancers that have known altered Ca_V expression or function.

Methylation of the CACNA1A gene is apparently associated with several cancers. In ovarian clear cell adenocarcinoma, increased methylation of CACNA1A was found to be linked with significantly reduced progression-free survival (Ho et al. 2012). Moreover, in lung cancer, CACNA1A has been identified as a novel tumour suppressor whose methylation was likely to result in adenocarcinoma (Castro et al. 2010). In contrast, non-methylation status of CACNA1A is reportedly associated with triple-negative breast cancer (Branham et al. 2012).

$Ca_V2.2$

CACNA1B is reported to be expressed in prostate and breast cancer (Wang et al. 2015). As discussed above, N-type $Ca_V2.2$ channels are co-expressed with Ca_V1 and Ca_V3 channels in neuroblastoma cells (Kito et al. 1999; Sousa et al. 2013). A number of papers present pharmacological data supporting the functional expression of Ca_V 2.2 channels (Reeve et al. 1994; Reuveny and Narahashi 1993; Lambert et al. 1990; Andres et al. 2013; Morikawa et al. 1998; and Sher et al. 1996) although at the time of some of the earlier studies, it may not have been possible to determine the subtype expressed. Sousa et al. (2013) found three splice variants of $Ca_V2.2$ in SH-SY5Y human neuroblastoma cells and in functional experiments observed effects from inhibition of $Ca_V2.2$ channels.

$Ca_V2.3$

R-type channels (CACNA1E) are reported to be significantly overexpressed in oesophageal and uterine cancer

(Wang et al. 2015) although data on functional expression are not yet available. CACNA1E is overexpressed in childhood kidney cancer Wilm's tumours, predominantly in the nuclei, and is associated with increased risk of relapse (Natrajan et al. 2006). Overexpression of $Ca_V3.2$ in HEK cells activated an MeK/ERK5/Nur77 pathway and may indicate a novel non-canonical role in cancer progression, which is a property of $Ca_V1.2$ and $Ca_V1.3$ channels (see below).

$Ca_V2.3$ channels are also involved in FSH-stimulated ovarian cancer cell growth (Li et al. 2007), which occurs via a cAMP-independent activation of ERK and is sensitive to the $Ca_V2.3$ channel inhibitor, SNX-482. Functional $Ca_V2.3$ channels have been demonstrated in pancreatic cancer cells (Bon-1) where channel activity is coupled to IGF-1 signalling and secretion of chromogranin A (Mergler et al. 2003, 2005). A recent study of somatic mutations in patients with non-small-cell lung cancer exposed to severe air pollution compared with patients not exposed to pollution found that the most frequent mutations were related to calcium signalling, notably including CACNA1E (Yu et al. 2015). This finding shows that CACNA1E and potentially other Ca_V channel genes can acquire mutations and act as drivers for certain cancers in contrast to having altered expression as a consequence of mutations elsewhere.

$Ca_V3.1$

Choi et al. (2014) reported anti-proliferative and apoptotic activities of a T-type calcium channel antagonist, BK10040, in human lung adenocarcinoma (A549) and pancreatic cancer (MiaPaCa2) cells. Consistent with this, expression of CACNA1G in lung cancer has been reported by Wang et al. (2015). In a related pharmacological study, the putative T-channel blocker KYS05090 induced autophagy- and apoptosis-mediated cell death in human lung adenocarcinoma A549 cells (Rim et al. 2014) and while it decreased intracellular Ca^{2+} levels, it was not found to directly cause cell death. The authors reported generation of reactive oxygen species and reduced glucose uptake and while the drug may have potential in lung cancer treatment, it might be independent of $Ca_V3.1$ activity. In a drug screening study on ovarian cancer cells, KYS05090 induced apoptosis, perhaps confirming the functional expression of T channels (Jang et al. 2013).

CACNA1G is highly expressed in human laryngeal squamous cell carcinoma tissues and experimental cell lines (Yu et al. 2014); moreover, siRNA techniques and the Ca_V3 channel blocker mibefradil inhibited proliferation and arrested cell cycle progression. Lu et al. (2008) screened a panel of oesophageal cancer cell lines and found gene expression of $Ca_V3.1$, $Ca_V3.2$ and $Ca_V3.3$; the latter also confirmed by Wang et al. (2015). Functional expression of T currents was confirmed by patch-clamp experiments and their role in cancer hallmarks was demonstrated by the reduction of proliferation by mibefradil or by siRNA. As discussed above, $Ca_V3.1$ is co-expressed in SH-SY5Y human neuroblastoma cells along with other Ca_V channels and accessory subunits (Sousa et al. 2013).

$Ca_V3.2$

Neuroendocrine differentiation of prostate cancer cells is an important mechanism for the development of poor prognostic tumours and is known to involve increased expression of functional $Ca_V3.2$ channels (Gackiere et al. 2008). In LNCaP cells, neuroendocrine differentiation evoked by androgen-reduced medium or cAMP increased the proportion of cells expressing $Ca_V3.2$ channels (Weaver et al. 2015a; Mariot et al. 2002), which were characterised with patch clamp, pharmacological blockers and siRNA (Mariot et al. 2002). $Ca_V3.2$ activity may act to stimulate secretion of mitogens and induce phenotypic change (Mariot et al. 2002; Fukami et al. 2015). It has also been shown that functional coupling between BK and $Ca_V3.2$ channels may act to drive proliferation of prostate cancer cells (Gackiere et al. 2013). The involvement of the tumour microenvironment in the upregulation of $Ca_V3.2$ in neuroendocrine differentiation is shown in recent work by Weaver et al. (2015b) where IL-6 significantly increased $Ca_V3.2$ protein expression but did not affect mRNA expression, indicative of a post-transcriptional mechanism. Interestingly IL-6 alone did not increase the expression of functional channels in the membrane but co-stimulation by IL-6 and the cAMP agent (forskolin) did increase functional channel expression. The development of a neuroendocrine morphology was prevented by $Ca_V3.2$ inhibition in IL-6-stimulated cells confirming the channels' role in this phenotype.

Ca_V channels may also be functionally expressed in leukaemia and lymphoma cell lines. Mibefradil reduced cell growth via decreasing proliferation and promoting apoptosis linked with Ca^{2+} release from the endoplasmic reticulum (Huang et al. 2015) indicating that these channels also participate in haematological malignancies.

The breast cancer cell line MCF-7 expresses $Ca_V3.1$ and $Ca_V3.2$ (Taylor et al. 2008a; Ranzato et al. 2014; Squecco et al. 2015), which seem to be involved in proliferation. In live-cell Ca^{2+}-imaging experiments, Ca_V3 channel blockers inhibited Ca^{2+} transients confirming functional Ca^{2+} influx through these channels. Inhibition or knockdown of Ca_V channels inhibited MCF-7 proliferation but not that of non-cancer breast epithelial cells; moreover, gene expression of $Ca_V3.1$ and $Ca_V3.2$ was only found in rapidly growing non-confluent cells compared with confluent cells (Taylor et al. 2008b).

$Ca_V3.2$ channel overexpression in glioblastoma multiform tumours is apparently associated with cell survival and resistance to therapy (Valerie et al. 2013). Inhibition or knockdown of Ca_V3 channels was found to reduce cell viability and clonogenic survival and also induced apoptosis. Similar effects were not found with L-channel inhibition confirming that $Ca_V3.2$ channels may represent novel targets for treatment of glioblastomas.

$Ca_V3.3$

There are few studies reporting CACNA1I in cancer; however, Wang et al. (2015) found overexpression of the gene in breast, sarcoma and oesophageal cancers. A study of colon, breast and prostate cancer cells subjected to increased extracellular pressure reported that T-type $Ca_V3.3$ channels modulated pressure-stimulated proliferation in all of the cells studied (Basson et al. 2015).

Non-canonical functions of Ca_V1 channels

As highlighted above, in addition to transport of Ca^{2+}, Ca_V channels also have non-canonical functions (Fig. 1). $Ca_V1.2$ and $Ca_V1.3$ α subunits have carboxyl terminus regions that can be cleaved not only modifying the remaining pore subunit (Gerhardstein et al. 2000; Gao et al. 2000, 2001) but also conferring functions in regulation of transcription when the c terminus translocates to the nucleus. Wei et al. (1994) found that removal of up to 70 % of the $Ca_V1.2$ carboxyl terminus increased current density by facilitating coupling between voltage-dependent gating and channel opening, resulting in increased channel open probability. Cleavage of the c-terminus of $Ca_V1.2$ has been shown to generate a transcription factor termed CCAT (Gomez-Ospina et al. 2006). Overexpression of the CCAT fragment resulted in altered expression of a number of proteins including the ion channels TRPV4 and KCNN3. Therefore, in addition to Ca^{2+} flux driving cancer-related signalling, the $Ca_V1.2$ c-terminus can further contribute by modulating or recruiting the expression of other channels to drive a cancer phenotype. Cleavage of the $Ca_V1.3$ c-terminus in atrial myocytes is reported to induce its translocation to the nucleus where it acts as a transcription factor, regulating the expression of SK2 channels (Lu et al. 2015). In addition, expression of a number of other proteins was altered including immunoglobulins, transcription factors and myosin light chain. Further work is required to define the genes regulated by actions of the c-terminus of VGCCs in different tissues and subsequent effects on cancer progression.

Interestingly, VGCCs are known to have a number of protein interaction sites along their amino acid sequence, which allows for interaction with proteins that influence gene transcription such as CREB, NFAT, calmodulin and MEK (see the review by Barbado et al. 2009), therefore enabling ion channels to indirectly control transcription of genes that are known to be involved in cancer development and progression (Xiao et al. 2010; Mancini and Toker 2009; Berchtold and Villalobo 2014).

Potential of repurposing Ca_V drugs for cancer therapy

Given the functional expression of Ca_V channels in several cancers and their confirmed role in Ca^{2+} transport, the use of CCBs may be beneficial in treating the disease. Many of the CCBs used in experimental models are FDA approved for the treatment of hypertension, epilepsy, chronic pain, etc. Such drugs could potentially be repurposed to treat cancer; moreover, epidemiological evidence describes cancer risk in the context of CCB use for other conditions. Given the ability of CCBs to target multiple Ca_V channels, further pre-clinical research is required to determine whether an effect on in vivo tumours would occur.

A number of epidemiological studies have investigated whether CCBs confer benefits or disadvantages in cancer patient cohorts. Several investigations report that the use of CCBs for other conditions may be correlated with a reduced risk of prostate cancer (Debes et al. 2004; Fitzpatrick et al. 2001; Lever et al. 1998; Rodriguez et al. 2009). Furthermore, other investigations have shown that CCB use is associated with significantly reduced prostate tumour aggressiveness and development of advanced disease (Kemppainen et al. 2011; Poch et al. 2013). Similar studies in breast cancer (Saltzman et al. 2013) have yielded mixed reports of CCBs with one study showing a significant reduction in breast cancer risk (Fitzpatrick et al. 1997), another reporting a trend of risk reduction (Fryzek et al. 2006) and other studies reporting no association (Bergman et al. 2014; Li et al. 2003, 2013, 2014; Chen et al. 2014b, 2015; Devore et al. 2015). In colorectal, lung and colon cancer, a number of studies of CCBs have shown no beneficial correlation (Boudreau et al. 2008; Michels et al. 1998).

It is perhaps not surprising that the epidemiological data are not yet definitive as tissue cancers are a heterogeneous group of diseases with distinctive molecular subtypes. Stratification of patient data, e.g., in prostate cancer by the presence of the TMPRS2-ERG fusion status (Tomlins et al. 2008) where it is known that CACNA1D is highly overexpressed, may show benefits of CCB use prior to development of aggressive disease. If that were to be the case, the molecular pathological diagnosis of prostate tumours may be beneficial to stratify patients towards CCB treatment in addition to standard of care therapy.

The Ca_V3 channel inhibitor, mibefradil, was previously FDA approved and used in clinical practice (Ertel and Clozel 1997) but was quickly withdrawn because of serious toxicity arising from effects on other transporters through adverse interactions with beta-blockers, digoxin, verapamil and diltiazem (Mullins et al. 1998). Other CCBs are well tolerated such as the diuretic amiloride, which also targets Ca_V3 (Tang et al. 1988), and it may be beneficial in the treatment of cancers where Ca_V3 is functionally overexpressed.

Drug repurposing could be enhanced by strategies that improve potency, selectivity and toxicity, for example the creation of a prodrug from linking the drug to a non-toxic promoiety (Karaman 2014). This strategy facilitates drug targeting to specific tissues with the benefits of reducing toxicity and improving selectivity by releasing the drug only at the target tissue site, e.g., tumour. A similar approach has been used with the sarcoplasmic/endoplasmic reticulum calcium adenosine triphosphatase (SERCA) pump inhibitor, thapsigargin, which has been linked to prostate-specific membrane antigen to direct it to prostate cancer cells (Denmeade et al. 2012). A similar approach could be used for drugs that target specific VGCCs that are expressed in tumour cells but that would have adverse effects in non-tumour cells. While this approach would take longer to reach clinical trial compared with simply repurposing existing drugs, advantages in reduced toxicity and better access to tumour cells would provide additional therapeutic benefits.

Conclusions

The pre-clinical science and expression data reviewed here indicate that VGCCs are overexpressed in many cancers and that in the majority of cases these are functional channels, facilitating Ca^{2+} transport and homeostasis. The potential of CCBs in cancer treatment, in addition to chemotherapy, surgery and radiation therapy, has not yet been fully investigated through either prospective clinical trials or retrospective epidemiological cohort analysis. Repurposing of CCBs for the benefit of cancer patients therefore presents an attractive opportunity to improve human health.

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