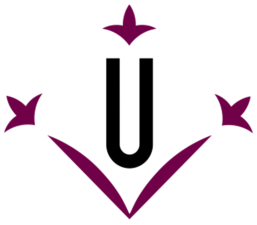

TITLE: T-type Ca^{2+} channels: T for targetable

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ABBREVIATION LIST: CaM, Calmodulin. CaN, Calcineurin. CDK, Cyclin dependent kinase. GBM, Glioblastoma multiforme. ER, Endoplasmic reticulum. ERK, Extracellular signal-regulated kinase. GSC, Glioma stem cells. FDA, Food and Drug Administration (U.S). HIFs, hypoxia inducible factors. IHC, Immunohistochemistry. MAPK, Mitogen activated kinase. K(Ca^{2+}) channels, Ca^{2+}-activated K^+ channels. MEK, Mitogen-activated protein kinase kinase**.** mTOR, Mammalian target of rapamycin. NFAT, Nuclear factor of activated T-cell. pCREB, cAMP response element binding protein.TCGA, The Cancer Genome Atlas. TTCC, T-type Ca^{2+} channels. UPR, Unfolded Protein Response. VEGF, Vascular Endothelial Growth Factor. V_m, Membrane potential.

Abstract

In the last decade TTCC have been unveiled as key regulators of cancer cells biology and thus have been proposed as chemotherapeutic targets. Indeed i*n vitro* and *in vivo* studies indicate that TTCC pharmacological blockers have a negative impact on the viability of cancer cells and reduce tumor size, respectively. Consequently mibefradil, a TTCC blocker approved in 1997 as an antihypertensive agent but withdrawn in 1998 because of drug-drug interactions, was granted 10 years later the orphan drug status by the FDA to investigate its efficacy against brain, ovary and pancreatic cancer. However, the existence of different channel isoforms with distinct physiological roles, together with the lack of selective pharmacological agents, has hindered a conclusive chemotherapeutic evaluation. Here we review the available evidence on TTCC expression, value as prognostic markers and effectiveness of their pharmacological blockade on cancer cells *in vitro* and in preclinical models. We additionally summarize the status of clinical trials using mibefradil against glioblastoma multiforme. Finally, we discuss the future perspectives and the importance of further development of multidisciplinary research efforts on the consideration of TTCC as biomarkers or targetable molecules in cancer.

Introduction

T-type Ca^{2+} channels (TTCC) were first reported by Hagiwara and colleagues in the 1970s in voltage-clamped starfish eggs (1). These channels could be distinguished from other voltage-gated Ca^{2+} channels (VGCC) on the basis of their fast activation and inactivation at negative potentials and slow deactivation kinetics. Because of these peculiarities and small single channel conductance, they were later coined as T-type Ca^{2+} channels (T for transient and tiny, TTCC) or low voltage-activated (LVA) channels, in contrast with the high voltage-activated channels (HVA) comprising the rest of VGCC families. It took quite a few years before the molecular cloning of the first member of the TTCC family, α1G (2), termed $Ca_v3.1$ by the nomenclature established in 2000 (3). This was shortly followed by the molecular identification of the two other members of TTCC, α1H ($Ca_v3.2$)(4) and α1I ($Ca_v3.3$)(5).

TTCC were initially linked to membrane excitability, such as cardiac pacemaker potentials (6) and neuron oscillatory firing (7)(8). TTCC are present in central and peripheral neurons, and abnormalities in their expression or function have been linked to a range of neurological diseases, including absence seizures, epilepsies and neuropathic pain (9)(10). Nonetheless, TTCC provide a key pathway for Ca^{2+} entry in non-excitable cells, and eventually their expression was found to be enhanced during the G1/S transition in proliferating cells (11)(12).

G1 to S phase transition requires Ca^{2+} influx through multiple Ca^{2+} channels at the plasma membrane (13). As cells re-enter the cell cycle in early G1, Ca^{2+} elevations promote the activation of AP-1 (Fos/Jun), cAMP-responsive element binding, and nuclear factor of activated T-cell (NFAT) transcription factors, which control the expression, assembly or stability of cyclin/cyclin dependent kinase (CDK) complexes essential for progression to the S phase (14) (Fig. 1).

The contribution of TTCC to cell cycle control is based on their distinct biophysical properties. From a closed-deactivated state, TTCC activate by weak membrane depolarization, which is rapidly followed by adoption of a closed-inactivated (refractory) state. At the steady-state, overlap between activation and inactivation potential ranges leaves a small fraction of TTCC open, enabling sustained inward Ca^{2+} currents (termed *window* currents) that may regulate Ca^{2+}-sensitive processes (15)(16). Membrane potential (V_m) is a key regulator of cell cycle and is subject to fluctuations in proliferating cells (17). Of note, V_m is hyperpolarized in the G1 and S phases, corresponding to the activation or increased expression of different K^+ channels, including Ca^{2+}-activated K^+ ($K(Ca^{2+})$) channels which form functional tandems with TTCC (7)(15). G1/S hyperpolarization could lead to increased Ca^{2+} window currents, or TTCC availability, especially in cells with more depolarized mean V_m, such as stem or cancer cells. The involvement of TTCC in cell cycle progression promoted by growth factors was modelled by Gray and colleagues (12): production of inositol triphosphate triggers Ca^{2+} release from the endoplasmic reticulum (ER), activating $K(Ca^{2+})$ channels. The ensuing membrane hyperpolarization removes TTCC inactivation, facilitating a Ca^{2+} influx that, upon Ca^{2+} binding to S100 proteins, inhibits the p53/p21 pathway to pass the G1/S restriction point. However, the signaling mechanisms that control cell cycle downstream of TTCC-mediated Ca^{2+} entry do not appear to be limited to p53 inactivation. Proven transducers of TTCC activity are calmodulin (CaM)and downstream effector calmodulin kinase II (CaMKII) (18). Activation of $Ca_v3.1$ heterologously expressed in HEK293 cells has been shown to transiently activate the Ras/MEK/extracellular signal-regulated kinase (ERK) pathway (19), whereas TTCC blockade inhibits the PKB/Akt pathway in GBM, being both signaling routes involved in G1/S progression (20). It is also known that $Ca_v3.2$ regulates the calcineurin (CaN)/NFAT pathway through both Ca^{2+} entry and direct binding to CaN, to induce cardiac hypertrophy (21). In addition, Ca^{2+} influx via $Ca_v3.2$ regulates the expression of the Sox9 transcription factor by CaN/NFAT activation during tracheal chondrogenesis (22)**.** Furthermore, $Ca_v3.1$-deficient T helper cells showed a reduced nuclear translocation of NFAT, in turn leading to a decreased secretion of Granulocyte-macrophage colony-stimulating factor and unveiling a role for TTCC in lymphocyte differentiation (23)**.** Thus and notwithstanding the proven role of TTCC in G1/S progression, the expression of TTCC can also be associated to cell cycle exit (Fig.1).

TTCC as prognostic markers in cancer

Increased basal Ca^{2+} influx and remodeled Ca^{2+} signaling pathways may contribute to tumor progression by enhancing proliferation, promoting invasiveness and conferring chemotherapeutic resistance (24)(25). Hence, important questions are whether TTCC are differentially expressed in cancer cells, and if the TTCC signature has prognostic value.

Available data indicates that TTCC expression levels depend on cancer type, stage and TTCC isoform (Table 1). Immunohistochemical staining (IHC) showed that both $Ca_v3.1$ and $Ca_v3.2$ expression were increased in tumoral vs normal ovarian tissue (26). According to Human Protein Atlas, 82% of glioblastoma multiforme (GBM) biopsies expressed $Ca_v3.1$ and 27% expressed $Ca_v3.2$. The same database indicates that all prostate cancer samples expressed $Ca_v3.2$, while 75% expressed $Ca_v3.1$. The expression of $Ca_v3.3$ was not determined (27). Maiques and colleagues performed IHC against TTCC comparing normal skin, melanocytic nevi and different types of melanoma (28). TTCC immunoexpression increased gradually from normal skin to common naevi, dysplastic naevi and melanoma samples, with differences in the distribution of isoforms. Particularly, $Ca_v3.2$ was highly expressed in metastatic compared to primary melanoma. Positive correlations were found between $Ca_v3.2$, proliferative and hypoxia markers, and between $Ca_v3.1$, autophagy markers and the BRAFV600E mutation. Furthermore, $Ca_v3.2$ transcripts and protein were highly expressed in a subset of GBM tumors enriched in glioma stem cells (GSC) (20), consistent with a previously described role for this isoform in stemness (29). However, the expression of the $Ca_v3.1$ isoform, which was previously shown at the mRNA level in a vast array of GSC lines (30), was not investigated.

Nonetheless, both up- and down-regulation of TTCC can become cancer's molecular signature. Phan and colleagues performed a bioinformatics analysis on the expression of TTCC transcripts in >4000 cancer tissue samples by accessing Oncomine, a microarray database (31). The three TTCC isoforms exhibited variable levels in several cancer subtypes, including over- and under-expression when compared to normal tissues (Table 1).

The prognostic relevance of the TTCC gene signature in cancer is a crucial question. An *in silico* genomic analysis of The Cancer Genome Atlas (TCGA) database revealed that disease-free and overall survival correlated inversely with expression of $Ca_v3.1$ and $Ca_v3.2$ in melanoma (28). Another study using the TCGA database revealed alterations of the $Ca_v3.2$ gene in 15/136 GBM samples, and these cases presented a trend towards shorter overall survival (20).

Querying the Kaplan-Meier plotter database, Fornaro and colleagues performed a correlation study between TTCC expression in solid tumors and patient survival. In gastric cancer patients, the expression of $Ca_v3.1$ was associated to an extended overall survival, whereas $Ca_v3.2$ (best single predictor) and $Ca_v3.3$

were associated with poorer outcomes. This trend was repeated for lung cancer, while in ovarian cancer patients $Ca_v3.1$ and $Ca_v3.2$ swapped the sign of their correlations with overall survival (32).

Thus, overexpression of specific TTCC isoforms appears to have a protective effect on specific cancer types and stages. Conversely, DNA aberrant methylation of the CACNA1G gene (encoding $Ca_v3.1$ channels) has been found in 18-35% of different human primary tumors, including pancreatic (33), hepatic (34), gastric, colorectal and acute myelogenous leukemia (35)(36). The promoter region of CACNA1G is a target for the CpG island methylator phenotype, which implies the inactivation of multiple tumor suppressor genes (35)(36)**.** The inactivation of CACNA1G may play a role in cancer development by favoring proliferation and/or avoiding apoptotic or autophagic pathways, but few studies have addressed these questions (Table 1).

Effects of TTCC blockade/gene silencing in cancer cells: *in vitro* studies

The expression of TTCC in cancer cells was first reported in retinoblastoma Y79 cells shortly after their molecular identification (37). The notion that it was possible to halt cancer cell proliferation or induce cancer cell death by inhibiting TTCC, was built up in the following years through *in vitro* studies on a wide range of cancer cells.

Reduced Proliferation

Numerous studies have shown that TTCC pharmacological blockade or gene silencing reduce the proliferation of cancer cells (12)(38). However, only a few provided insights on the pathways triggered by TTCC blockade/silencing. Lu and colleagues reported that pharmacological inhibition of TTCC with mibefradil reduced cell proliferation via p53-dependent upregulation of CDK inhibitor p21 (39), halting G1/S progression (Fig.1). Further, mibefradil-induced activation of the p38-mitogen activated kinase (MAPK) pathway caused p53 accumulation, resulting in cell cycle arrest (and death) of colon cancer cells (40).

Inhibition of neuroendocrine differentiation

Unlike most cancer types, neuroblastoma can undergo a spontaneous complete regression through neuronal-like differentiation (41). Chemin and colleagues demonstrated that either pharmacological blockade of TTCC or interfering with the expression of $Ca_v3.2$ (using antisense nucleotides) prevented cAMP-induced neuritogenesis of neuroblastoma-glioma NG108-15 cells and HVA Ca^{2+} channel expression, indicating a dual role of TTCC in promoting morphological changes and membrane excitability at early

stages of neuronal differentiation (42). Moreover, H_2S- induced differentiation of NG108-15 cells involved the phosphorylation of Src kinase and was abolished by ascorbic acid (a proven inhibitor of $Ca_v3.2$) and by mibefradil (43). Other than Src activation, Chemin and colleagues found that Ca^{2+} influx through TTCC promotes neuroblastoma differentiation by an autocrine mechanism (44).

$Ca_v3.2$ channels also proved to be relevant for neuroendocrine differentiation of human prostate cancer cells (45)(46)(47). During this process, prostate cancer cells develop neurite-like processes and secrete diverse mitogenic factors with paracrine or autocrine actions (47). Opposite to neuroblastoma, differentiation of prostate epithelial cells is associated with an increased aggressiveness of prostate tumors, adoption of an androgen-refractory phenotype and poor prognosis (48). Two recent works confirmed the mediation of $Ca_v3.2$ channels in the differentiation of prostate cancer LNCaP cells subject to physiologically relevant stimuli: sodium butyrate increased the expression of $Ca_v3.2$ channels at the mRNA and protein level, and their pharmacological blockade decreased the number and length of neurite-like processes and cell viability (46). The same research group showed that interleukin-6 up-regulated $Ca_v3.2$ channels by a posttranscriptional mechanism. Again, pharmacological blockade of TTCCs limited neurite number and extension (47).

Decreased cell survival

2013 was a turning point in the consideration of the physiological roles of TTCC in cancer cells. Dzigielewska and colleagues reported a dual effect of mibefradil on reducing proliferation and inducing caspase-dependent apoptosis of colon cancer cells, by p38-MAPK activation and p53 upregulation (40). Our research group proved that the structurally unrelated TTCC blockers mibefradil and pimozide halt melanoma cell proliferation at the G1/S transition and induce the intrinsic apoptosis pathway with activation of caspases-3 and -9 (49). Death occurred after induction of the unfolded protein response to ER stress, followed by subsequent blockade of constitutive autophagy. siRNA-mediated silencing of $Ca_v3.1$ and $Ca_v3.2$ isoforms exerted similar effects, demonstrating that TTCC play a role in Ca^{2+} homeostasis maintenance and in sustaining basal macroautophagy. The sequence of events linking TTCC blockade/silencing and autophagy deregulation has only been partially elucidated. Huang and colleagues showed that mibefradil and its derivative NNC-55-0396 exerted a dual role on leukemia cells viability, by promoting both G1/S arrest and apoptosis, that was preceded by ER Ca^{2+}-release and depolarization of the mitochondrial membrane (50). Earlier this year, Niklasson et al. performed a drug screening assay on GSC and found that, among different disruptors of Ca^{2+} signaling network, TTCC and K(Ca^{2+}) channel blockers decreased their viability (30). Transcriptomics and proteomics analyses revealed that, upon channel

blockade, the GSC plasma membrane depolarized, intracellular Na^+ increased and Na^+-dependent transport was reduced, leading to nutrient starvation and cell death.

Nutrient starvation is a stimulus for macroautophagy by activating AMP-activated protein kinase and/or inhibiting the mTOR complex (51). Accordingly, KYS05090, a dihydroquinazoline with TTCC-blocking properties, induced autophagy and apoptosis in lung carcinoma A549 cells through reactive oxygen species generation and subsequent inhibition of glucose uptake (52). Nevertheless, autophagy deregulation by TTCC inhibition appears to be double edged. It is known that Ca^{2+} is required for phagosome-lysosome function (53); in this scenario, it is tempting to speculate that TTCC inhibition could prevent the influx of Ca^{2+} necessary for autophagolysosomal formation, *de facto* blocking macroautophagy at a late step, as observed for the effects of mibefradil in melanoma cells (49).

TTCC blockade/knockdown can also lead to apoptosis by inactivating Ca^{2+} signaling pathways relevant for cell survival. Valerie and colleagues showed that, in addition of inhibiting proliferation, mibefradil or siRNA-mediated $Ca_v3.1$/$Ca_v3.2$ gene silencing induced the apoptotic death of GBM cells through dephosphorylation of pro-survival Akt and cleavage of caspase-3 and -7 (54). This pathway can also be triggered in ovarian cancer cells, resulting in reduced levels of anti-apoptotic survivin (55). Remarkably, these works demonstrated that TTCC inhibition also sensitizes cancer cells to the chemotherapeutic of choice (temozolomide for GBM and carboplatin for ovarian cancer), thus establishing the grounds for the use of TTCC blockers in combined therapies.

Similarly, Zhang and colleagues have recently shown that mibefradil treatment reduced the viability of GSC, partly due to inhibition of pro-survival Akt/mTOR pathways and upregulation of p27 and BAX proteins (20). A further RNA-seq transcriptomic analyses on GSC found that mibefradil attenuated the expression of several oncogenes and promoted the expression of different tumor suppressor genes, proving that the signaling pathways stemming from TTCC blockade can be complex and intricate.

Together, these works indicate that the role of TTCC spans beyond the control of cancer cell cycle into the regulation of cancer cell homeostasis, such that their pharmacological blockade or gene silencing deregulates Ca^{2+} dependent physiological processes pivotal for cell survival (Fig. 2).

Effects of TTCC blockade/gene silencing on tumor growth: *in vivo* studies

While TTCC blockade/gene silencing has shown to decrease the viability of cancer cells *in vitro*, albeit with notable exceptions regarding $Ca_v3.1$ knockdown, these strategies needed validation in preclinical models before consideration of TTCC targeting in clinical assays. 3D tumor growth implies a hypoxic microenvironment and altered focal/cell-cell adhesions that shape tumor progression, invasiveness and

sensitivity to therapeutic agents (56)(57). Chronic hypoxia triggers the transcriptional upregulation of $Ca_v3.2$ channels in several cell types, mediated by hypoxia inducible factors (HIFs) (58)(59). Thus, the TTCC gene signature in cancer cells is likely to depend on O_2 availability and HIFs activity. Intriguingly, the connection between TTCC and HIFs appears to be bidirectional: exposure to mibefradil reduced hypoxia-induced HIF1α and HIF2 in GSC (20). Furthermore, TTCC blockade using different pharmacological agents, or $Ca_v3.2$ silencing, resulted in a reduced stability of HIF1α protein and expression of Vascular Endothelial Growth Factor (VEGF), ultimately inhibiting GBM-induced angiogenesis (60).

Pioneer studies by Jung and colleagues evaluated the antitumor activity of KYS05090 in a mouse lung adenocarcinoma A549 xenograft, which slowed down tumor growth upon intravenous (61) or oral administration (62). Another 3,4-dihydroquinazoline able to block TTCC, KYS05047, demonstrated antitumor efficacy in the same xenograft model when administered orally (63).

Other groups studied the effect of mibefradil or NNC-55-096 against solid tumors growing *in vivo*. In a xenograft model of ovarian cancer, HO8910 cells developed smaller tumors when co-injected with NNC-55-096 (26). A similar approach was performed on a U87 GBM xenograft model (60). In consonance with *in vitro* results, intraperitoneal injection of NNC-55-0396 delayed tumor growth by inhibiting angiogenesis with a concomitant reduction of angiogenetic regulators (such as HIF-1α, VEGF and Platelet-endothelial cell adhesion molecule).

More elaborated protocols of mibefradil administration have been performed against diverse subcutaneous and intracranial GBM xenografts. Keir et al. designed a chemotherapeutic strategy in which mibefradil was first administered in order to synchronize GBM cell cycle at the G1/S boundary, then withdrawn followed by administration of alkylating agent temozolomide (64). The rationale behind this approach, termed *interlaced therapy*, was that mibefradil exposure would reduce the time for DNA repair systems to act against temozolomide-induced damage. Indeed, this combined therapy enhanced the efficacy of best single treatment (temozolomide), increasing overall survival by 18-68% depending on tumor types, implant location and treatment schedule.

In addition, TTCC pharmacological blockade might synergize with radiotherapy, a common therapeutic tool for GBM. A study on rats carrying intracranial C6 glioma implants, showed that intraperitoneal injection of mibefradil and simultaneous radiosurgery slowed tumor growth and extended median survival from 35 (radiosurgery alone) to 43 days. The benefits of initiating mibefradil treatment 1 week prior to radiotherapy were even stronger, achieving 52 days of median survival (65). These results suggest that the response to mibefradil in conjunction with ionizing radiation is also schedule-dependent.

Recently, administration of mibefradil inhibited the growth of GSC-derived intracranially-implanted GBM murine xenografts, and sensitized tumors to temozolomide treatment (20). In this study, two cycles of mibefradil (oral) and/or temozolomide (intraperitoneal) were concurrently administered. IHC revealed that proliferation marker Ki67 and stem cell marker SOX2 decreased, whereas astrocyte marker GFAP and caspase 3 increased in mibefradil-treated tumors. Data also showed that single treatments inhibited tumor growth by a similar magnitude, and that the combined treatment inhibited tumor growth in an additive fashion. Consistently, both mibefradil and temozolomide alone significantly prolonged animal survival, which was further extended with the combined treatment.

Clinical trials:

Early results attained in murine xenografts encouraged the enrollment of high-grade GBM patients in clinical trials in which TTCC are pharmacologically targeted with mibefradil, a drug with a well-known pharmacokinetic and toxicity profile (66). *Cavion Pharma* LLC (formerly *Tau Therapeutics LLC*), a pharmaceutical company focused on drug development and on the repurposing of mibefradil for oncology and neurological disease, performed in 2012 a dose escalation study to assess the safety of mibefradil dihydrochloride in 30 healthy patients, which rendered only mild and self-limited adverse effects (NCT01550458). This was followed by the launch of a second phase I study, in conjunction with the National Cancer Institute, to assess the efficiency and optimal dosage of mibefradil sequentially administered in combination with temozolomide in patients with recurrent GBM (NCT01480050). The results for this trial indicate that the therapy was generally well tolerated (67)**.** A third trial sponsored by the same company in collaboration with Yale University has also been conducted (NCT02202993, 2014-2017). This was a dose-escalation study to determine the safety and the maximum tolerated dose of mibefradil combined with hypofractionated radiation in patients with recurrent GBM, with no results published to date.

Conclusion:

Pharmacological blockade of TTCC reduces the viability of cancer cells *in vitro* and tumor growth *in vivo*. Preclinical results spearheaded the first clinical trials employing mibefradil in combined therapies against GBM. Yet, a compelling evaluation of TTCC as prognosis markers and/or targetable proteins in cancer will require a comprehensive characterization of the TTCC molecular signature, and a deeper knowledge of the cell signaling pathways stemming from TTCC activation/inhibition. Individual TTCC isoforms play different roles in cancer pathophysiology, but this notion is hampered by the absence of selective pharmacological

modulators. The expression of $Ca_v3.1$, which showed a positive correlation with autophagy markers, is predominant in some cancer types, while epigenetically silenced in others. The expression of $Ca_v3.2$, which increases in hypoxic conditions, has been associated to cancer stemness, aggressiveness and metastasis. The expression of $Ca_v3.3$ across cancer tissues remains largely unexplored, in spite of current evidences for a negative correlation with survival of gastric, lung and ovarian cancer patients. In addition to cancer, TTCC are currently under the scope of different biomedical fields, including neurological and cardiovascular disease. Multidisciplinary research efforts are bound to facilitate the development of isoform-specific tools, and will hopefully galvanize fine-tuned approaches for different cancer types and stages.

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Table 1: TTCC signature and value as prognostic markers in cancer

TTCC isoform	Tumor type	Prognostic	Expression level	Assay	References
$Ca_v3.1$	ovarian	n.d.	increased protein *	IHC	26
$Ca_v3.2$	ovarian	n.d.	increased protein *	IHC	26
$Ca_v3.1$	GBM (82% samples), prostate (75% samples)	n.d.	n.d.	IHC	27
$Ca_v3.2$	GBM (27% samples), prostate (100% samples)	n.d.	n.d.	IHC	27
$Ca_v3.1$	melanoma	negative	increased protein *	IHC	28
$Ca_v3.2$	melanoma	negative	increased protein *, **	IHC	28
$Ca_v3.2$	subset of GBM enriched in GSC	negative	increased mRNA*and increased protein*	RT-PCR and WB	20
$Ca_v3.1$	sarcoma, lung, uterine, prostate, breast carcinoma, rectosigmoid carcinoma ovarian, renal, brain, bladder, mantel cell lymphoma, colorectal carcinoma	n.d.	increased mRNA* decreased mRNA*	MA, Oncomine database	31
$Ca_v3.2$	renal, sarcoma, gastrointestinal stroma brain, ovarian, bladder, breast	n.d.	increased mRNA* decreased mRNA*	MA, Oncomine database	31
$Ca_v3.3$	breast, liposarcoma, esophageal adenocarcinoma GBM, anaplastic oligondendroglioma	n.d.	increased mRNA* decreased mRNA*	MA, Oncomine database	31
$Ca_v3.1$	gastric, lung ovarian	positive negative	n.d.	MA, Kaplan-Meier database	32
$Ca_v3.2$	gastric, lung ovarian	negative positive	n.d.	MA, Kaplan-Meier database	32
$Ca_v3.3$	gastric, lung ,ovarian	negative	n.d.	MA, Kaplan-Meier database	32
$Ca_v3.1$	pancreatic, hepatic, gastric, colorectal, acute myelogenous leukemia (18-35% samples)	n.d.	hypermethylation	MSP	33-36

*relative to untransformed tissue; ** metastatic vs. primary tumors

MA: microarray analysis; WB: Western blot; MSP: methylation-specific PCR

Legend to Figure 1

TTCC form functional complexes with K(Ca^{2+}) channels in the plasma membrane and trigger signaling pathways that may favor cell cycle progression or differentiation, depending on cell type and context. The figure combines a selection of relationships identified in different studies for TTCC-mediated signaling (green arrows) and effects of TTCC inhibition (red crosses/arrows), including activation of Cyclin/CDK complexes and CaM-dependent phosphorylation/dephosphorylation events. Arrow tips indicate whether the modulation is positive (pointed) or negative (round). Dashed lines indicate ion fluxes/transport across the plasma membrane.

Legend to Figure 2

TTCC-mediated signaling (green arrows) and effects of TTCC inhibition (red crosses/arrows) on cell survival/apoptosis. The figure combines a selection of relationships identified in different studies. Arrow tips indicate whether the modulation is positive (pointed) or negative (round). TTCC inhibition may induce apoptosis by PKB/Akt dephosphorylation or by activation of the p38-MAPK-p53 axis. TTCC/K(Ca^{2+}) blockade may also cause plasma membrane depolarization and compromise Na^{+}-dependent nutrient transport, in turn inducing the unfolded protein response (UPR) and the amino acid response (AAR), which may convey into apoptosis or autophagy. A question mark '?' indicates that the contribution of TTCC to these Ca^{2+}-dependent processes is speculative.

cell cycle

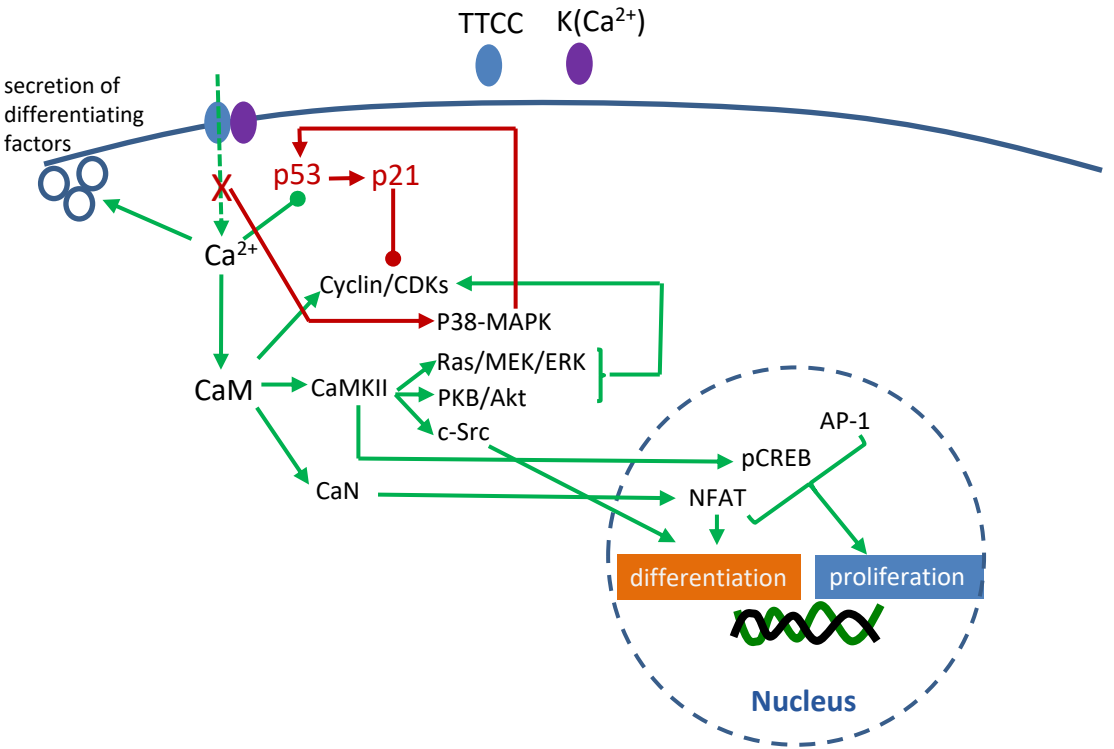

Figure 1 Sallán et al.

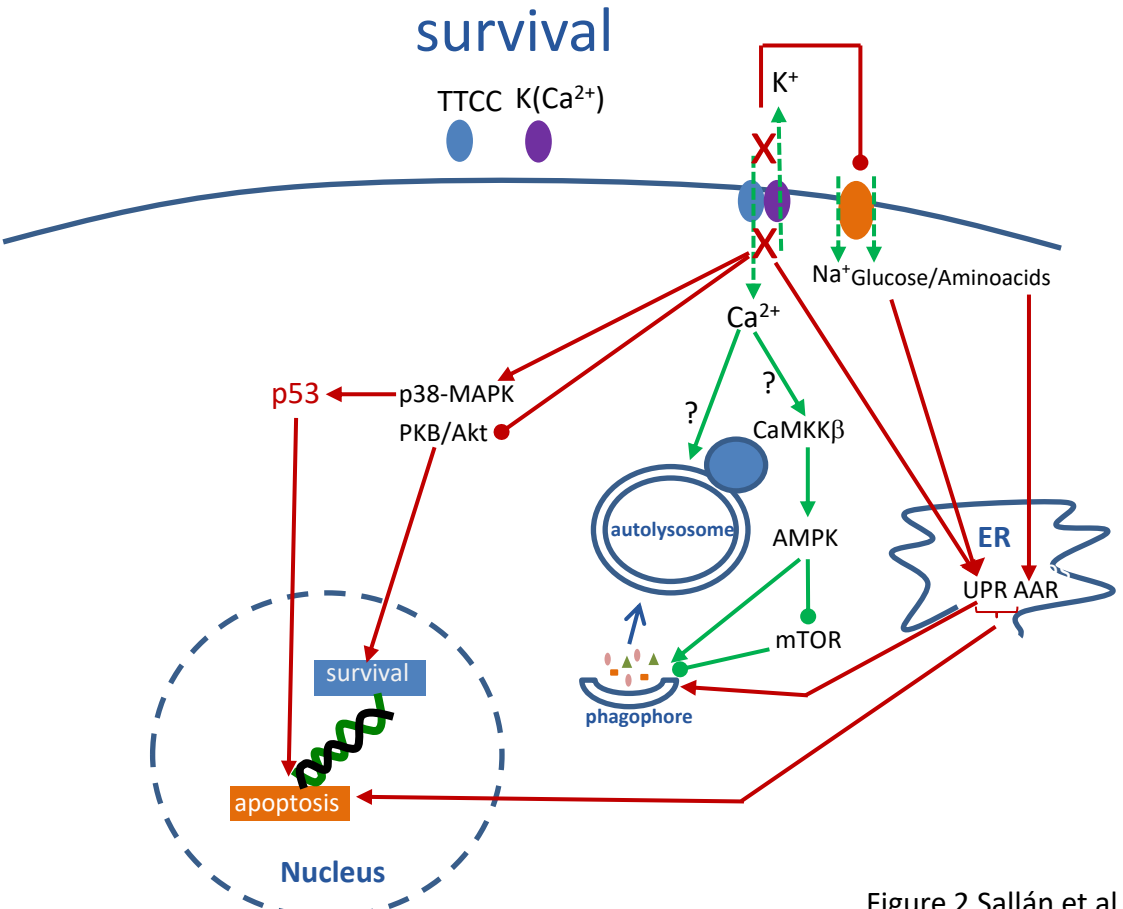

Figure 2 Sallán et al.