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COMMENTARY

Functional role of T-type calcium channels in tumour growth and progression: prospective in cancer therapy

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T-type Ca^{2+} channels represent a specific channel family overexpressed in different types of tumours. Their involvement in controlling the proliferation, angiogenesis and invasion of tumour cells, has been partially clarified. The article by Zhang *et al.* in this issue of *BJP* provides the first evidence of anti-tumoural effects of endostatin (ES) in U87 glioma cells. He demonstrated that ES or mibefradil (a L/T-type calcium channel blocker), reduces the proliferation and migration of U87 glioma cells in a T-type Ca^{2+} channel-dependent manner. However, the difference in the blocking effect of mibefradil on T-type calcium channel expression as compared with its ability to inhibit proliferation and migration, supports the idea of a broader T/L-type-independent effect of the mibefradil blocker. Overall, these findings provide new insights for the future development of a novel class of anti-T-type calcium channel blockers in the therapy of glioblastoma.

LINKED ARTICLE

This article is a commentary on Zhang *et al.*, pp. 1247–1260 of this issue. To view this paper visit http://dx.doi.org/10.1111/j.1476-5381.2012.01852.x

Abbreviations

ES, endostatin; GBM, glioblastoma multiforme

Calcium plays a key role in intracellular signalling and controls many different cell processes such as proliferation, differentiation, growth, cell death and apoptosis. Thus, alterations in calcium signalling can cause defects in cell growth and invasion and are associated with certain types of cancer. A number of research groups have suggested a potential role for voltage-activated Ca^{2+} channels, in particular T-type, in the regulation of tumour growth and progression.

Molecular biology studies have expanded the repertoire of these Ca^{2+} channels revealing three main subfamilies of α_1 subunit called Ca_v1, Ca_v2 and Ca_v3. The third subfamily contains three members that are called T-types: $Ca_v3.1$ (α_{1G}), $Ca_v3.2$ (α_{1H}) and $Ca_v3.3$ (α_{1I}). The unique low voltage–dependent activation/inactivation and slow deactivation of T-type Ca^{2+} channels suggest that they play a direct role in regulating $[Ca^{2+}]_i$, especially in non-excitable tissues, including some cancerous cells (Lory *et al.*, 2006; Panner and Wurster, 2006). At low voltages, T-type Ca^{2+} channels produce the so-called 'window current' at appropriate values of membrane potential, that results in a sustained inward calcium current carried by the portion of channels that are not completely inactivated. This regulation of Ca^{2+} homeostasis allows T-type Ca^{2+} channels to control cell proliferation and differentiation. Therefore, loss of T-type Ca^{2+} channel control may lead to aberrant cell growth and tumour progression.

A role for these channels in tumour cell proliferation has been reported in breast, brain, colorectal, gastric, hepatic and prostate tumours, leukaemic cells, retinoblastoma cells and phaeochromocytoma cells (Lory *et al.*, 2006; Panner and Wurster, 2006). Human breast adenocarcinoma MCF-7 cell line exhibits α_{1G} and α_{1H} T-type Ca^{2+} channel mRNA and T-current transiently (Taylor *et al.*, 2008). Human prostate cancer epithelial cells (LNCap) have also been shown to display increased T-type Ca^{2+} channel (α_{1H}) current and mRNA. Finally, overexpression of T-type α_1-subunit genes, either α_{1H} alone or together with α_{1G} and α_{1I}, has been demonstrated in human oesophageal tumours, as compared with the normal counterpart that shows a lower α_1 expression. In

accordance with the T-type Ca^{2+} channel expression, their blockade diminished the proliferation of oesophageal cancer cells through p53-dependent $p21^{CIP1}$ up-regulation (Lu *et al.*, 2008).

The mechanisms underlying the *in vivo* anti-tumoural action of T-type Ca^{2+} channel antagonists are less well understood. In athymic nude mice implanted with MCF-7 breast cancer cells, injection of mibefradil (0.5 mg·100 μL^{-1}, twice a week) at tumour sites resulted in marked tumour degeneration and necrosis (Taylor *et al.*, 2008). Furthermore, local intra-cerebral micro-infusion of endostatin (ES) improved treatment efficiency and survival in a xenograft orthotopic human glioblastoma multiforme (GBM) model.

The expression of T-type Ca^{2+} channels can vary depending on tumour stage. For instance, differentiation of epithelial prostate cancer cells into more aggressive neuroendocrine cells that express functional T-type Ca^{2+} triggers the release of growth factors stimulating the proliferation of neighbouring prostate cancer cells. Similarly, T-type Ca^{2+} channels mediate the release of growth factors in pheochromocytomas; in retinoblastoma cells the decreased proliferation was accompanied by a reduced expression of T-type channels mRNA and decreased T-currents.

In this issue of *British Journal of Pharmacology*, Zhang *et al.* (2012), by using ES, a proteolytic fragment of collagen XVIII, explored the functional role of T-type Ca^{2+} channels in U87 glioma cells and in HEK-293 and CHO heterologous cell expression systems.

Conflicting observations are available on the expression of α1 subunits of T-type Ca^{2+} channels $Ca_v3.1$ (α_{1G}), $Ca_v3.2$ (α_{1H}) and $Ca_v3.3$ (α_{1I}) in U87 glioma cells. Recently, Panner *et al.* (2005) have demonstrated a decrease in the expression of the α_{1G} and α_{1H} subunit associated with decreased proliferation. By contrast, using real-time PCR and electrophysiological methods, Lu *et al.*, (2005) failed to detect T-type channel mRNA and T-currents. In the present paper, Zhang *et al.* (2012) confirmed Panner's data showing that U87 cells express all of the three α1 subunits of T-type Ca^{2+}channels. By using transfected HEK293 or CHO cells, they found that only $Ca_V3.1$ and $Ca_V3.2$, but not $Ca_V3.3$ or $Ca_V1.2$ (L-type), channel currents were significantly inhibited by ES.

Zhang *et al.* (2012) showed that treatment with ES inhibited T-currents in U87 cells, whereas L-currents were not affected. Moreover, pretreatment of U87 glioma cells with the T-type Ca^{2+} channel blocker, mibefradil, or with the L-type blockers nifedipine or nimodipine, inhibited cell proliferation and migration. This inhibitory effect was associated with a hyperpolarizing shift in the voltage-dependence of inactivation. The authors also observed that inhibition of T-currents induced by ES was highly dependent on the inactivation state of the channel. ES-mediated hyperpolarization induced a shift of the steady-state inactivation curve (approximately –15 mV), whereas the activation of curve was not affected. Although it is unclear whether the hyperpolarizing shift of the steady-state inactivation curve would produce a significant modification in T-type 'window current', it is conceivable that it could depend on an increased number of channels remaining in the inactivated state after activation. Further studies are needed to address how making fewer T-type Ca^{2+} channels available for opening mechanistically, contributes to the inhibitory effect of ES on glioma cellular responses.

Mibefradil (Posicor) was the first mixed T/L channel blocker to be marketed for its ability to block T-currents. Unfortunately, it is metabolized by cytochromes P450 3A4 and 2D6, leading to drug–drug interactions. Mibefradil can exert non-specific anti-proliferative effects because it accumulates and is hydrolyzed inside the cells and the resulting metabolites can block T-type Ca^{2+} channels. Thus, to avoid these problems, in the present paper, data obtained with mibefredil were also evaluated by using NNC 55–0396, a mibefradil nonhydrolyzable analogue without L-type Ca^{2+} channel efficacy (Panner and Wurster, 2006).

ES or mibefradil inhibits fibronectin-induced migration of U87 glioma cells. Similarly, results were induced in fibrosarcoma cells, where mibefradil suppressed T-type Ca^{2+}-mediated Ca^{2+} spikes, waves, cell motility and invasive properties (Huang *et al.*, 2004). The magnitude of the inhibitory effect of mibefradil on the motility of U87 cells was higher than that induced by ES, suggesting that this blocker also shows broader T/L-type-independent effects.

The findings by Zhang *et al.* (2012) also demonstrate that the ES-mediated effects on the induced proliferation and migration of U87 glioma cells is not mediated by G-proteins or tyrosine-kinase signalling pathways, raise a crucial question as to the nature of the signalling pathways involved in ES inhibition of Ca^{2+} T-type channels and how they regulate $Ca_V3.1/2$ activity. T-channel activity can be modulated by hormones and neurotransmitters acting through signalling intermediates such as protein kinases A and C, calmodulin-dependent protein kinase II, tyrosine kinase, G-proteins and lipid derivatives such as arachidonic acid. Recent reports suggest a role for PKC and ERK pathways in T-type channel activation. Phorbol-12-myristate-13-acetate potently enhances, although to different extents, the current amplitude of $Ca_v3.1$, $Ca_v3.2$ and $Ca_v3.3$ channels, via PKC activation (Park *et al.*, 2006). At present, it is not completely understood whether the PKC-mediated stimulation might depend on direct phosphorylation of Ca_v3 channels or represent an indirect consequence of phosphorylation of associated targeting, anchoring or signalling protein(s). Ciliary neurotrophic factor increases the expression and currents of T-type Ca^{2+} channels by triggering JAK/STAT and ERK signalling pathways (Trimarchi *et al.*, 2009). Further studies are necessary to elucidate this issue.

An expansion of the list of ion channels implicated in cancer development is expected. It is difficult to ascribe tumour development to the malfunction of a single ion channel. For instance, regulation of K^+ or TRP channels can affect the membrane potential, which in turn regulates the window currents mediated by T-type Ca^{2+} channels. However, as in many cases there are already known pharmacological modulators (blockers and activators) of ion channels, the identification of a single defective ion channel in a particular cancer could provide a ready-to-go therapeutic approach (Gray and Macdonald, 2006).

In conclusion, the study of Zhang and colleagues highlighted the therapeutic potential of ES via targeting T-type calcium channels for the treatment of human GBM. Recently, a sequential administration of a T-channel blocker to synchronize cells at the G_1/S checkpoint of the cell cycle before

the administration of chemotherapy (Interlaced Therapy™), has been reported by Tau Therapeutics (LLC Charlottesville, USA). Thus, in GBM, the administration of mibefradil before temozolomide has been found to overcome the resistance of GBM patients to temozolomide and significantly increases their life-span. However, because T-type Ca^{2+} channels are normally expressed in the brain, heart and endocrine tissues of the human body, the potential side effects of the specific channel blockers must to be considered for therapeutic applications.

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