



HAL
open science

TRP channels in cancer

Natalia Prevarskaya, Lei Zhang, Greg Barritt

► **To cite this version:**

Natalia Prevarskaya, Lei Zhang, Greg Barritt. TRP channels in cancer. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, 2007, 1772 (8), pp.937. 10.1016/j.bbadis.2007.05.006 . hal-00562788

HAL Id: hal-00562788

<https://hal.science/hal-00562788>

Submitted on 4 Feb 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

TRP channels in cancer

Natalia Prevarskaya, Lei Zhang, Greg Barritt

PII: S0925-4439(07)00113-5
DOI: doi: [10.1016/j.bbadis.2007.05.006](https://doi.org/10.1016/j.bbadis.2007.05.006)
Reference: BBADIS 62732

To appear in: *BBA - Molecular Basis of Disease*

Received date: 23 May 2007
Accepted date: 25 May 2007



Please cite this article as: Natalia Prevarskaya, Lei Zhang, Greg Barritt, TRP channels in cancer, *BBA - Molecular Basis of Disease* (2007), doi: [10.1016/j.bbadis.2007.05.006](https://doi.org/10.1016/j.bbadis.2007.05.006)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

TRP Channels in Cancer

Natalia Prevarskaya ^{a,b}, Lei Zhang ^c and Greg Barritt ^d

^a Inserm, U800, Equipe Labellisee par la Ligue Contre le Cancer, Villeneuve d'Ascq F-59650, France

^b Universite de Lille 1, Villeneuve d'Ascq F-59650, France

^c Office of Chemical Safety, Therapeutic Goods Administration, PO Box 100, Woden, ACT, Australia

^d Department of Medical Biochemistry, School of Medicine, Flinders University, Adelaide, South Australia, 5001, Australia

Address for Correspondence and Proofs:

Professor Greg J. Barritt

Department of Medical Biochemistry

School of Medicine

Flinders University

GPO Box 2100

Adelaide SA 5001

Australia

Tel: +61 8 8204 4260

Fax: +61 8 8374 0139

Email: Greg.Barritt@flinders.edu.au

Abstract

The progression of cells from a normal differentiated state in which rates of proliferation and apoptosis are balanced to a tumorigenic and metastatic state involves the accumulation of mutations in multiple key signalling proteins and the evolution and clonal selection of more aggressive cell phenotypes. These events are associated with changes in the expression of numerous other proteins. This process of tumorigenesis involves the altered expression of one or more TRP proteins, depending on the nature of the cancer. The most clearly described changes are those involving TRPM8, TRPV6 and TRPM1. Expression of TRPM8 is substantially increased in androgen-dependent prostate cancer cells, but is decreased in androgen independent and metastatic prostate cancer. TRPM8 expression is regulated, in part, by androgens, most likely through androgen response elements in the TRPM8 promoter region. TRPM8 channels are involved in the regulation of cell proliferation and apoptosis. Expression of TRPV6 is also increased in prostate cancer and in a number of other cancers. In contrast to TRPM8, expression of TRPV6 is not directly regulated by androgens. TRPM1 is highly expressed in early stage melanomas but its expression declines with increases in the degree of aggressiveness of the melanoma. The expression of TRPV1, TRPC1, TRPC6, TRPM4, and TRPM5 is also increased in some cancers. The level of expression of TRPM8 and TRPV6 in prostate cancer, and of TRPM1 in melanomas, potentially provides a good prognostic marker for predicting the course of the cancer in individuals. The *Drosophila melanogaster*, TRPL, and the TRPV1 and TRPM8 proteins, have been used to try to develop strategies to selectively kill cancer cells by activating Ca^{2+} and Na^{+} entry, producing a sustained increase in the cytoplasmic concentration of these ions, and subsequent cell death by apoptosis and necrosis. TRPV1 is expressed in neurones involved in sensing cancer pain, and is a potential target for pharmacological inhibition of cancer pain in bone metastases, pancreatic cancer and most likely in other cancers. Further studies are required to assess which other TRP proteins are associated

with the development and progression of cancer, what roles TRP proteins play in this process, and to develop further knowledge of TRP proteins as targets for pharmaceutical intervention and targeting in cancer.

Keywords: Cancer, TRP channels, Intracellular Ca^{2+} , Sensory nerves, Cell death, Hormones.

1. Introduction

The processes involved in the transformation of normal cells to tumorigenic cells and in tumour progression are complex and are only partly understood (reviewed in [1-2]). At the macroscopic and histological levels, tumorigenesis involves the transition from normal cells to hyperplastic, dysplastic, neoplastic and then to metastatic cells. This transition is caused by the accumulation of mutations in certain key signalling proteins, encoded by oncogenes and tumour suppressor genes, and the formation and selection by evolution of those cells which can more aggressively compete in their local environment and, in the case of metastatic cells, in the environments of other organs. Among the signalling pathways altered in tumorigenesis those which enhance cell proliferation and inhibit apoptosis are some of the most important. For example, during the development of prostate cancer, where in the initial stages cell proliferation is dependent on androgens, a small number of cells become insensitive to androgens and resistant to apoptosis (reviewed in [3,4]).

Many proteins in cancer cells exhibit increased or decreased expression compared to their levels of expression in normal cells. Some of these proteins, chiefly those encoded by oncogenes and tumour suppressor genes, play key roles in tumorigenesis and in the development of metastases, while others, most likely including those involved in intracellular Ca^{2+} homeostasis (reviewed in [3,5]), are associated with cancer progression but are not causative in further development of the tumour and/or malignant cells (reviewed in [1]). Most

cancers are heterogeneous with respect to rates of growth and degrees of aggression. This most likely reflects the fact that, for a given cancer, there can be different combinations of oncogenes and tumour suppressor genes which are mutated, different sequences in which these mutations occur, and variations in the time over which the mutations accumulate [1].

Several members of the TRP family of Ca^{2+} - and Na^{+} -permeable channels (reviewed in [6-7]) show altered expression in cancer cells. The most studied are TRPM8, TRPV6, TRPM1 and TRPV1 (Table 1). To date, most changes involving TRP proteins do not involve mutations in the TRP gene but rather increased or decreased levels of expression of the normal (wild type) TRP protein, depending on the stage of the cancer. It is not yet possible to say whether these changes in TRP expression are central steps in the progression of the cancer or are secondary to other changes, although the latter is most likely. Irrespective of the answer to this question, several TRP proteins may prove to be valuable markers in predicting the progress of cancers and are potential targets for pharmaceutical treatment [8,12,13,20,25]. Also, since a number of TRP channels are involved in sensory neurons, TRP channels play a role in sensing pain in cancer (reviewed in [7]) and may be targets in strategies to alleviate this pain [26,27].

The aim of this review is to summarise the observations reported to date of changes in TRP protein expression associated with cancer and its progression, to comment on what this may mean in terms of cell physiology and pathology, and to identify where TRP proteins can be potential targets for diagnosis and therapeutic intervention. We are now only beginning to understand how TRP proteins may be involved in cancer. Furthermore, attempts to understand this involvement are being made against the background of incomplete, and in some cases very incomplete, knowledge of the normal physiological functions of TRP proteins.

2. TRPM8

Cold/menthol-sensitive TRPM8 of the “melastatin” TRP subfamily has recently emerged as an important player in carcinogenesis, whose real significance, however, is only beginning to unfold. TRPM8 is a so-called “cold” receptor belonging to the melastatin (TRPM) subfamily of TRP channels and is activated by cooling temperatures and menthol. Out of the broad array of thermal receptors cold/menthol-sensitive TRPM8 seems to be one of the most intriguing while still remaining the least studied. The intrigue comes from the fact that aside from sensory neurons, in which the role of TRPM8 in mediating cold-evoked excitation is fairly well established [28,29], it is most abundantly expressed in the prostate – a tissue, which is not involved in temperature-dependent functions. In fact, TRPM8 was first cloned from the human prostate as prostate-specific gene [12], even before its role in the cold sensation was established.

TRPM8 is a cation channel and in physiological conditions it facilitates Ca^{2+} and Na^{+} entry across the plasma membrane, leading to its depolarisation. In sensory neurons TRPM8 is activated by cold stimuli. However, despite the numerous hypotheses raised, the mechanisms of TRPM8 activation in organs not exposed to ambient temperatures, and in particular in prostate gland, remain poorly understood. Indeed, a model ascribing TRPM8 activation to cold/menthol-induced shifts of the channel voltage dependence towards physiological membrane potentials has been proposed [30]. Although attractive in its explanation of the temperature sensitivity, this model seems to be insufficient to account for TRPM8 activation in the tissues not exposed to any essential temperature variations. Moreover, prominent rundown of TRPM8 activity in excised patches [30,31] raises the possibility that some endogenous ligands might be necessary for the channel activation. Phosphatidylinositol 4,5-bisphosphate (PIP_2) has been found to be such a factor, as it was capable not only restoring menthol-

activated TRPM8 current after its rundown in excised patches, but also of activating the current independently of menthol [32]. These results suggest the importance of this endogenous lipid signalling molecule in sustaining the TRPM8 function. The functional importance of the PIP₂-dependent TRPM8 gating has thus become evident in the cold sensation since any of the well-known PIP₂ depletion scenarios (e.g., activation of G_{q/11}-phospholipase C (PLC) coupled receptors or Ca²⁺-dependent activation of some PLC isoforms) would limit TRPM8 activation by shifting its gating towards lower temperatures or higher voltages. However, the possibility of channel activation (rather than desensitization) with the involvement of PIP₂ seems more remote.

Recently a novel concept of TRPM8 activation has been proposed [33]. This mechanism involves stimulation by the Ca²⁺-independent phospholipase A₂ (iPLA₂) thereby employing chemical rather than physical (temperature change) signalling. The authors have found that the active substances in the TRPM8 activation were lysophospholipids, lysophosphatidylcholine and lysophosphatidylinositol, which are produced upon iPLA₂ stimulation. The fact that receptor-mediated stimulation of PLC will potentially produce oppositely directed effects on TRPM8 – inhibition via decreasing PIP₂ levels and activation via IP₃-mediated store depletion – seems puzzling. It can be explained assuming differential roles for the two lipid messenger pathways: the PIP₂ primarily controlling TRPM8 *desensitization* [32] with the lysophospholipids providing the main chemical *activation* input. Such an iPLA₂-dependent mechanism of TRPM8 activation has been confirmed in dorsal root ganglia neurons, suggesting its ubiquitous character [34]. Interestingly, several reports [35,36,37] indicate that TRPM8 is expressed not only in the plasma membrane (PM) (called PMTRPM8), as initially anticipated, but also in the endoplasmic reticulum (ER) membrane (called ERTRPM8), where it operates as an ER Ca²⁺ release channel involved in the activation of store-operated calcium entry (SOCE)

in response to cold/menthol stimulus. Since the depletion of intracellular Ca^{2+} stores is known to be causally associated with the stimulation of the iPLA_2 [38], catalysing the production of lysophospholipids, both PMTRPM8 and ERTRPM8 could be activated by an iPLA_2 -related mechanism, however, by store-independent or store-dependent mode of activation, respectively.

While remaining at moderate levels in a normal prostate, TRPM8 expression strongly increases in prostate cancer. For this reason it has been proposed to be a pro-oncogenic actor in prostate cancer cells [12]. Other non-prostatic primary human tumours (breast, colon, lung, and skin) also become highly enriched in TRPM8, although it is virtually undetectable in corresponding normal tissues [12]. Thus, even this initial information strongly pointed to much broader roles of TRPM8 beyond cold sensation, especially in the prostate and during carcinogenesis. The role of TRPM8 in organs not exposed to ambient temperatures, and especially in the prostate gland, remains a gnawing mystery. However, the data accumulated in the last three years allows the formulation of several hypotheses.

TRPM8 could function as a cold sensor in the prostate [39], but may also be involved in other functions such as ion and protein secretion, regulation of proliferation and/or apoptosis in prostate epithelial cells (for review see [40]). Nevertheless, the secretion of citric acid, prostate-specific antigen (PSA), acid phosphatase, several enzymes, lipids and other products is the major function of apical epithelial prostate cells. Therefore, considering the specific TRPM8 expression in these cells, the potential role of this channel in secretion has been suggested [41]. Furthermore, the recent study of Mergler et al has confirmed this hypothesis on neuroendocrine pancreatic tumour cells [15]. The authors have shown that TRPM8 activation increases the secretion of neurotensin in these cells.

In normal prostate, *trpm8* gene expression seems to be directly controlled by androgen receptors [35,41] positioning it as a primary androgen-response gene [41]. Single-cell RT-PCR and immunohistochemical experiments conducted on primary human prostate cancer cells have shown that TRPM8 is mainly expressed in androgen-dependent, apical secretory epithelial cells, and that its expression becomes down-regulated in cells losing the androgen receptor activity and regressing to the basal epithelial phenotype [41]. Mature prostate epithelial cells are non-proliferative cells which are highly sensitive to apoptotic stimuli. (Due to the specific regulation of the expression of genes belonging to the *Bcl-2* family, anti-apoptotic *Bcl-2* gene expression is repressed whereas pro-apoptotic *Bax* gene expression is stimulated by androgen receptors [42-44]).

In prostate cancer tumours, a significant difference in the expression level of TRPM8 mRNA between malignant and non-malignant tissue specimens has been detected [13]. This was comparable to the currently used prostate cancer marker, PSA, thus, qualifying TRPM8 as its potential competitor in prostate cancer diagnosis and staging. A significant difference in TRPM8 expression between human benign prostate hyperplasia and prostate cancer tissues is also obvious at protein level [14]. According to Tsavaler's hypothesis defining *trpm8* as an oncogene [12], TRPM8 over-expression and over activity in circumscribed, androgen-dependent prostate cancer may be correlated to the higher rate of growth of these cells compared to normal ones [45,46]. During the transition to androgen independence, TRPM8 is lost in a xenograft model of prostate cancer and also in prostate cancer tissue from patients treated preoperatively with anti-androgen therapy, suggesting that its loss may be associated with a more advanced form of the disease [47]. It has been demonstrated that LNCaP cells resistant to anti-androgen bicalutamide treatment displayed a reduced doubling time [14]. This

is correlated with a decreased expression of mRNA encoding the androgen receptor, TRPM8 and the Proliferating Cell Nuclear Antigen (PCNA), while anti-apoptotic Bcl-2 mRNA expression is increased. All these data reinforce the putative pro-proliferative role of TRPM8 in androgen-dependent prostate cancer cells. Finally, it has also been shown that both pharmacological activation of TRPM8 and siRNA-mediated TRPM8 silencing in LNCaP cells can decrease the cell viability [35], probably by perturbing the TRPM8-dependent intracellular Ca^{2+} homeostasis. However, it is still not clear whether TRPM8 involvement in cell viability is carried out through a pro-proliferative and/or an anti-apoptotic mechanism.

The results of the very recent study of Prevarskaya's team suggest that, depending on its intracellular localization, TRPM8 could potentially regulate both proliferation and apoptosis in prostate epithelial cells and, therefore, specific inhibition of either ER-TRPM8 or PM-TRPM8 may be considered depending on the stage and androgen-sensitivity of the targeted prostate cancer [48]. Using a combination of electrophysiology, Ca^{2+} imaging, molecular and cell biology approaches (in studies of primary cultures of normal and cancerous human prostate epithelial cells) this team has shown that only highly differentiated prostate epithelial luminal cells express functional plasma membrane TRPM8 (PM-TRPM8) channels. Importantly, prostate primary epithelial cancer cells obtained from *in situ* prostate cancer biopsies were characterized by significantly larger PM-TRPM8 -mediated current density than normal or benign prostate hyperplasia cells. This PM-TRPM8 activity was abolished in de-differentiated cells that had lost their luminal secretory phenotype. In contrast, ER-TRPM8 remained functional irrespective of the differentiation status of prostate cells. This differential regulation of TRPM8 activity has been explained by the complex regulation of ER-TRPM8 and PM-TRPM8 isoforms by androgen receptors.

In view of the fact that the role of TRPM8 in LNCaP cell survival has recently been demonstrated [35], the authors speculate that any shift in the balance between classical TRPM8 and ER-specific TRPM8 isoform expression may modify the Ca^{2+} signature, thus increasing the potential for either proliferation or apoptosis. Indeed, alterations in Ca^{2+} homeostasis increase proliferation [49-51], as well as induce differentiation [52] and apoptosis [53,54] (for review see [3]). Considering that ER Ca^{2+} content is known to regulate cancer cell growth, the finding that ER-TRPM8 is functional in de-differentiated prostate cancer cells with down-regulated androgen receptor provides new insight into the role of this channel in prostate cancer progression and may be of great importance in developing therapeutic strategies for metastasized prostate cancer.

3. TRPM1

TRPM1 (initially called melastatin) was discovered in B-16 mouse melanoma cell lines as a result of differential display analysis [8]. Heterologous expression of TRPM1 in HEK293 cells has been shown to lead to an increase in the cytoplasmic free Ca^{2+} concentration ($[\text{Ca}^{2+}]_{\text{cyt}}$) at the normal extracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_{\text{ext}}$), and a corresponding increase or decrease in $[\text{Ca}^{2+}]_{\text{cyt}}$ when $[\text{Ca}^{2+}]_{\text{ext}}$ was increased or decreased compared to the absence of such changes in control HEK cells [55]. This suggests that when heterologously expressed, TRPM1 is constitutively active (active in the absence of addition of any known activator). Ca^{2+} entry through TRPM1 is inhibited by La^{3+} and Gd^{3+} [55]. To date, no electrophysiological studies of TRPM1 have been reported. Several splice variants of TRPM1 have been detected, including one designated TRPM1-S (short, 500 amino acids). TRPM1-S interacts with full length TRPM1 (TRPM1-L, long, 520 amino acids) and there is some evidence to indicate that this interaction prevents the trafficking of TRPM1-L to the plasma membrane, and may play a role in regulation of Ca^{2+} entry through TRPM1 [55,56]. While it has been suggested that TRPM1

may be involved in the regulation of cell proliferation [25], the cellular and biological roles of TRPM1 are not well understood.

Studies of TRPM1 expression in B-16 mouse melanoma cell lines have shown that poorly metastatic variants, such as B-16-F1, express higher levels of TRPM1 mRNA compared to highly metastatic variants, such as B-16-F10 [8]. In specimens of human melanoma tissue, Duncan and colleagues, using *in situ* hybridisation, found high levels of TRPM1 expression in benign tissue (nevi), decreased expression in primary melanomas, and no detectable mRNA in metastatic melanomas [8]. TRPM1 mRNA expression appears to correlate with the state of melanocytic tumour progression, thickness of the tumour, and the degree of aggression, with low or undetectable TRPM1 mRNA expression in aggressive tumours [9-11].

These observations have led to the suggestion that the *trpm1* gene is a tumour suppressor [9-11]. It has also been suggested that the level of expression of TRPM1 mRNA may be a prognostic marker for the development of melanoma metastasis, and hence, may permit the prediction of the development of non-metastatic or metastatic melanomas [8,25].

Studies of the regulation of expression of TRPM1 in melanocytes and melanoma cells have provided evidence that TRPM1 expression is linked to the state of cell differentiation, and is regulated by the microphthalmia transcription factor (MIRF). Thus, the treatment of human pigmented metastatic melanoma cells with hexamethylene bisacetamide, an inducer of cell differentiation, led to an increase in TRPM1 mRNA expression [9]. Other studies have shown that the promoter region of the TRPM1 gene contains binding sites for microphthalmia, indicating that TRPM1 expression is likely to be regulated by MIRF. This transcription factor also regulates expression of a number of other melanoma cell proteins [57-59].

4. TRPV6

Studies with heterologously expressed TRPV6 have shown that this channel has a high selectivity for Ca^{2+} compared to Na^{+} [60]. Results of experiments with heterologously expressed TRPV6 using fura-2 to measure $[\text{Ca}^{2+}]_{\text{cyt}}$ suggest that TRPV6 is constitutively active, but under physiological conditions using perforated patch-clamping no constitutive current was observed [61,62]. Ca^{2+} entry through TRPV6 can be enhanced by a decrease in $[\text{Ca}^{2+}]_{\text{cyt}}$ or removal of extracellular divalent cations, leading to the conclusion that TRPV6 Ca^{2+} entry is regulated by both extracellular and intracellular Ca^{2+} [61,62]. Heterologous expression of TRPV6 in HEK293 cells leads to an increase in the rate of cell proliferation but it is not clear if this is due to enhanced Ca^{2+} entry through TRPV6 [63]. TRPV6 is expressed in the intestine, kidney, placenta and pancreas [64,65]. One of the biological functions of TRPV6 is to mediate transcellular Ca^{2+} movement across epithelial cells of the gut, kidney and placenta [24,65,66]. Consistent with this is the observation that 1,25-dihydroxy cholecalciferol (vitamin D) substantially increases TRPV6 expression [67].

When compared with normal tissue or cells, the expression of TRPV6 mRNA (measured using semi-quantitative or real time quantitative RT-PCR) and/or expression of the TRPV6 protein (measured by immunofluorescence) is substantially increased in prostate cancer tissue, and in human carcinomas of the colon, breast, thyroid, and ovary [20,21,23,64]. Increased expression of TRPV6 mRNA compared with that in normal cells is also observed in the LNCaP and PC-3 prostate cancer cell lines [23], in SW480 colorectal cancer cell lines [23] and in the K-562 chronic myelogenous leukaemia cell line [24]. Increased expression of TRPV6 is not observed

in pancreatic carcinoma and some other cancers [64] indicating that TRPV6 is not a general marker for tumorigenic cells [25,64].

More extensive studies of TRPV6 expression have been made in prostate cancer. In healthy and benign human prostate tissue the expression of TRPV6 mRNA is very low or is not detectable [20,64]. Studies with prostate cancer tissue obtained from biopsies or resections show substantial expression of TRPV6 mRNA which increases with the degree of aggressiveness of the cancer, assessed by the Gleason score (grading of the pathological stage) and the degree of metastasis outside the prostate [20,23]. These observations have led to the suggestion that the level of expression of TRPV6 could be used as a marker to predict the clinical outcome of prostate cancer [20,25].

As mentioned above, TRPV6 is expressed in the LNCaP human prostate cancer cell line. Prevarskaya's team have recently shown that TRPV6 is directly involved in the control of proliferation in LNCaP cells by decreasing: (i) proliferation rate; (ii) cells accumulation into S-phase of the cell cycle; and (iii) proliferating cell nuclear antigen (PCNA) expression [68]. This team has demonstrated that Ca^{2+} uptake into LNCaP cells is mediated by TRPV6, with the subsequent downstream activation of nuclear factor of activated T cells (NFAT).. The possible role of androgens in the regulation of TRPV6 mRNA expression remains unclear. Previous studies have shown that the androgen receptor agonist dihydrotestosterone inhibits TRPV6 expression while the androgen receptor antagonist bicalutamide increases TRPV6 expression [23,69,70]. However, TRPV6 expression has not been identified in androgen-insensitive prostate cancer cell lines DU-145 and PC-3 [20] and, moreover, Lehenkyi et al have shown that TRPV6 expression in LNCaP cells is regulated by androgen receptor, however in a ligand-independent fashion [68]. To date, little is known about whether the observed increased

expression of TRPV6 mRNA and protein in prostate cancer cells is associated with increased Ca^{2+} and Na^{+} entry through functional TRPV6 channels, and what the physiological and pathological consequences might be [25]. However, it has been shown from studies with LNCaP cells that TRPV6 is involved in the current activated by intracellular stores depletion [69-73] and in the Ca^{2+} uptake in prostate cancer cells [68].

5. Other TRP Proteins

Several other TRP proteins, TRPC1, TRPC6, TRPM5 and TRPV1, have been reported to be associated with tumorigenesis and the growth and progression of cancer cells. It is likely that further studies will reveal associations between other TRP proteins and cancer cells. In the transition from androgen-dependent to androgen-independent prostate cancer, the expression of TRPC1 is decreased [7]. This may have physiological and/or pathological consequences since it has been suggested that TRPC1 is a component of SOCs in prostate cancer cells [69,72,74]. In studies of primary human prostate cancer epithelial cells, in which TRPC6 expression was ablated using antisense hybrid deletion, Ca^{2+} entry, activation of NFAT and cell proliferation activated by α -adrenergic receptors were inhibited. It was suggested that Ca^{2+} entry through TRPC6 channels is required for NFAT activation and cell proliferation [51].

Suguro et al have studied the CD5+ subgroup of diffuse large B-cell lymphomas which comprise about 30% of non-Hodgkin's lymphomas [75]. CD5+ is associated with poor prognosis. TRPM4 mRNA expression was found to be increased in CD5+ lymphomas along with increased or decreased expression of a number of other genes. It was suggested that TRPM4 is one of several genes which constitute a "CD5 signature" which can be used as a prognostic clinical marker.

Indirect genetic evidence suggests that altered expression of TRPM5 may be associated with tumorigenesis. Alterations in region 11p15.5 of the human chromosome are known to be associated with Beckwith-Wiedemann syndrome and with a predisposition to neoplasias including Wilms' tumours, rhaboid tumours, and rhabdomyosarcomas (reviewed in [76]). In a study of candidate genes in the 11p15.5 region, Prawitt et al identified TRPM5 as one of the genes in this region of DNA [76]. Moreover, TRPM5 mRNA was found to be expressed in a large proportion of Wilms' tumours and rhabdomyosarcomas [76].

Increased expression of TRPV1 has been found to be associated with prostate cancer and cancers of the colon, pancreas and bladder [16-19]. Substantial TRPV1 expression has been observed in the LNCaP and PC-3 prostate cancer cell lines [19]. Other studies have shown that TRPV1 is expressed in the normal urothelium of the bladder. Moreover, the amount of TRPV1 decreased in cancerous urothelium with a progressive decrease in TRPV1 expression observed as the carcinoma progresses to a more aggressive stage and the cells become more de-differentiated [18].

Immunohistochemical studies have shown that TRPV1 is expressed in colon adenocarcinoma [16]. Moreover, it has been shown that the concentration of extracellular polyamines, a TRPV1 agonist, in tissues of the gastrointestinal tract increases during inflammation and cancer [77]. This raises the possibility that TRPV1 may be activated by polyamines in colon cancer, possibly contributing to cancer pain. TRPV1 may also play a role in cancer pain in pancreatic cancer. Thus, expression of TRPV1 is increased in pancreatic cancer and in pancreatitis. The extent of TRPV1 expression was found to be related to the intensity of pain reported by cancer patients, but this relationship was not found in patients with chronic pancreatitis [17].

6. TRP channels as potential pharmaceutical targets in cancer treatment

Two aspects of the properties of TRP proteins and the association of increased or decreased expression of a given TRP protein with cancer and the progression of cancer have been used to try to develop strategies to kill cancer cells. One aspect uses Ca^{2+} and Na^+ entry through TRP channels expressed in cancer cells which leads to a sustained high $[\text{Ca}^{2+}]_{\text{cyt}}$ and cytoplasmic Na^+ concentration ($[\text{Na}^+]_{\text{cyt}}$) which kills the cells by apoptosis and necrosis. This strategy requires the selective expression and activation of a given TRP channel in the targeted cancer cells. New strategies for killing cancer cells by activation of the apoptotic pathway are valuable, since for many cancer cells, including androgen-insensitive prostate cancer cells, the normal pathways of apoptosis are inhibited and the cells are resistant to apoptosis [78-81]. The other aspect makes use of the high expression of some TRP channels in cancer cells to provide a target for delivering a toxic payload (eg a radioactive nuclide or toxic chemical) to the cancer cells. Recognition of the TRP protein could be achieved through a tight-binding agonist or an anti-TRP antibody (reviewed in [82]).

There are several examples in normal physiology and pathophysiology where Ca^{2+} and Na^+ entry through TRP channels leads to cell death [83,84]. Some studies have extended this observation to use Ca^{2+} and Na^+ entry through TRP channels as a strategy to kill cancer cells. The mechanisms which lead to Ca^{2+} - and Na^+ -induced cell death by apoptosis and necrosis are complex. They probably include initiation of the intracellular pathway of apoptosis and pathways of necrosis by the sustained high $[\text{Ca}^{2+}]_{\text{cyt}}$ which likely leads to the activation of proteases, phospholipases, and the loss of mitochondrial function due to the uptake of large amounts of Ca^{2+} (Fig 1A) [53,78,85,86]. The sustained increase in $[\text{Na}^+]_{\text{cyt}}$ most likely

contributes to the initiation of necrosis by inducing cell swelling, the activation of proteases and phospholipids, and changes in membrane potential and ion transporters involved in volume regulation [87-89]. The strategy of using sustained elevated $[Ca^{2+}]_{cyt}$ to kill cells builds on earlier studies of Isaacs and others, which employed the SERCA inhibitor thapsigargin to release Ca^{2+} from the ER which, in turn, induces ER stress and activates sustained Ca^{2+} entry through SOCs [90].

Two “proof of principle” studies employed the *Drosophila melanogaster* photoreceptor TRPL channel to kill prostate cancer cells. When heterologously expressed in some animal cells, TRPL is constitutively active and permits the entry of Ca^{2+} and Na^+ [91-93]. TRPL was heterologously expressed in LNCaP and PC-3 prostate cancer cells under the control of the CMV promoter, a prostate specific androgen promoter construct, or the inducible doxycycline-sensitive Tet-On promoter system. Expression of TRPL led to constitutive Ca^{2+} entry, an increase in $[Ca^{2+}]_{cyt}$ and cell death by apoptosis and necrosis [87,89]. A similar strategy involving heterologous expression of TRPV1, which has the advantage of being activated by a ligand (e.g. capsaicin), has also been tested with prostate cancer cells (Fig 1B and C) (Zhang, L and Barritt, G J, unpublished results) (see also [94]). These strategies offer the potential to selectively kill prostate (and other) cancer cells. Strategies to provide selectivity include the use of prostate-specific promoters to selectively express the TRP channel in prostate cancer cells [87], and delivery of agonists as a “pro-drug”. For example, an inactive conjugate of the required TRP agonist with a peptide which is cleavable by the proteolytic activity of prostate specific antigen (Fig 1D) [90,95,96].

Studies with some other cancers also suggest that TRPV1 may be a useful target for killing cancer cells through a sustained increase in $[Ca^{2+}]_{cyt}$ and $[Na^+]_{cyt}$. Resiniferatoxin, an analogue

of capsaicin and an agonist of TRPV1, was shown to cause inhibition of mitochondrial function and induce apoptosis in pancreatic cancer cells, presumably via endogenous TRPV1 channels in the plasma membrane [17]. It was suggested that vanilloids might be used to treat pancreatic cancer [17].

Lignesti and colleagues tested the ability of various plant cannaboids, which bind to the cannaboid (CB) and TRPV1 receptors, to inhibit tumour cell growth [97]. Using a panel of tumour cell lines as well as a xenograft mouse model of breast cancer (MDA-MB-231 cells), they found that cannaboids, of which cannabidiol was the most potent, inhibited cell and tumour growth. They suggested cannaboids may act through CB2 receptors and the TRPV1 channels. Endogenous cannaboids play an important role in the neuronal control of the digestive tract [98], and Izzo and Coutts have suggested that the pharmacological administration of cannaboids, which, in part, act through TRPV1 channels could be used to treat colon cancer [98].

As discussed above, TRPM8 is expressed in prostate cancer cells, its expression decreases as the cancer progresses to a more metastatic state, and hence TRPM8 is considered potentially useful for both the diagnosis of prostate cancer and as a target for cancer therapy. The treatment of prostate cancer would be greatly enhanced by better prediction of the course of the disease, including the likelihood of the development of androgen insensitivity and metastases, and by new strategies to kill androgen-insensitive prostate cancer cells which, as mentioned above, are resistant to apoptosis [78-80].

Recently a menthol analogue and TRPM8 agonist, WS-12, has been synthesised and characterised. WS-12 has an affinity for the TRPM8 menthol binding site which is about 2,000

times higher than that for menthol itself [4]. Incorporation of a fluorine atom into WS-12 resulted in an analogue (WS-12F) which activated TRPM8 by 75% of the activation induced by WS-12 and retained a high affinity for TRPM8. It has been suggested that WS-12 and WS-12F offer potential possibilities in the detection of micro metastases and in killing prostate cancer cells. Thus, the incorporation of ^{18}F into WS-12 may permit radio-imaging of micro metastases, and/or the delivery of a radionuclide, which could kill target cells, to specific locations of cancer cells in both the prostate and in metastases [4].

7. TRP Channels as targets for the management of cancer pain

Pain is a major issue for many cancer patients, and an important consideration in the care of these patients. The TRPV1, TRPV3, TRPV4, TRPA1 and TRPM8 proteins are involved in the detection of a variety of painful stimuli by nociceptive tumours (reviewed in [7]). The results of several studies suggest that TRPV1 is a useful pharmacological target for the treatment of cancer pain. TRPV1 is expressed in nociceptors that detect multiple pain-producing stimuli including heat and extracellular H^+ (acid) (reviewed in [7]). Hartel et al have provided evidence that expression of TRPV1 mRNA is higher in pancreatic tissue in those patients who reported higher levels of pain from pancreatic cancer, and have suggested that the pharmacological application of vanilloids might be useful for the treatment of neurogenic pain in patients with pancreatic cancer [17]. In a mouse model of cancer pain, the TRPV1 antagonist capsazepine was found to inhibit hyperalgesia induced by tumour cell inoculation [99]. The authors suggested that altered TRPV1 expression may be responsible for the tumour-induced hyperalgesia.

In a mouse model of thermal hyperalgesia induced by tibial osteosarcoma, capsaizepine, a TRPV1 antagonist, and resiniferatoxin, a TRPV1 agonist, both inhibited osteosarcoma induced hyperalgesia [26]. This somewhat surprising result suggested an involvement of TRPV1 in osteosarcoma-induced hyperalgesia. Ghilardi et al found that TRPV1 is expressed in sensory neurons that innervate the mouse femur, and using an *in vivo* mouse model of bone cancer pain showed that the acute or chronic administration of a TRPV1 antagonist, or disruption of the TRPV1 gene led to a reduction of nocifensive behaviours [27]. The results of both these studies suggest that further evaluation of TRPV1 as a pharmaceutical target for the reduction of pain due to bone cancer metastasis will prove beneficial [26,27].

8. Conclusions.

From the discussion above the following conclusions can be drawn. (i) TRPM8, TRPM1, and TRPV6 are highly expressed in cancer cells and the amount of protein expressed changes with progression from normal to tumorigenic to metastatic cells. The expression of some other TRP channels, including TRPC1, TRPC6, TRPM5 and TRPV1 is also increased in cancer tissues. So far, there is no evidence to indicate that the expression of a given TRP protein is consistently increased or decreased in many different cancers, although further studies may show this is the case for some TRP proteins, for example, TRPM8. (ii) It has been suggested that TRPM8 and TRPV1 may be oncogenes and TRPM1 a tumour suppressor gene, but further experiments are required to test these hypotheses. (iii) Many of the physiological and pathophysiological roles of the TRP proteins which are expressed at high levels in cancer tissue are still to be elucidated. This task is presently made more difficult because, for some TRP channels, little is known of their functions in normal cells. The roles of TRP channels in cancer progression may involve changes in intracellular Ca^{2+} and Na^+ , although effects on Ca^{2+} would be complex since the expression of some TRP proteins increases while that of others decreases

as cancer progresses [25]. Another likely possibility is that some of the effects of TRP proteins in cancer cells are exerted by their interactions with other intracellular proteins [25]. Elucidation of the cellular functions of TRP channels in tumorigenesis and in cancer cells might be achieved by ablation of a given TRP protein, for example, by use of a non-functional TRP mutant [25]. (iv) TRPM8, TRPM1 and TRPV1 are potential diagnostic markers for the prognosis of tumour development especially the degree of tumour aggression, and are potential targets for pharmaceutical interventions. Such interventions may involve use of the TRP protein as a recognition site for antibody-mediated delivery of a toxic pay load, or possibly more directly through selective activation of the TRP channel to induce sustained Ca^{2+} and Na^{+} entry, and subsequent necrosis and apoptosis.

It is predicted that there will be considerable further progress in understanding changes in TRP channel expression in cancer cells in the next few years as knowledge of both the molecular events involved in cancer progression and the physiological functions of TRP channels increases. One of the main challenges will be gaining an understanding of whether the involvement of a given TRP channel is part of the causative mechanisms involved in cancer progression or is associated in a secondary manner with these mechanisms and changes.

Acknowledgements

Research conducted in the authors' laboratories which has contributed to this review is supported by grants from the National Health and Medical Research Council of Australia, the Flinders Medical Centre Foundation of South Australia; and Institut National de la Sante et de la Recherche Medicale, Ligue Nationale Contre le Cancer, and Association pour la Recherche Contre le Cancer.

References

- [1] Weinberg, R.A. Multi-step tumorigenesis in the biology of cancer. Garland Science Chapter 11 (2006) 399-461.
- [2] Gupta, G.P. and Massague, J. Cancer metastasis: Building a framework. *Cell* 127 (2006) 679-95.
- [3] Prevarskaya, N., Skryma, R. and Shuba, Y. Ca^{2+} homeostasis in apoptotic resistance of prostate cancer cells. *Biochem. Biophys. Res. Commun.* 322 (2004) 1326-35.
- [4] Beck, B., Bidaux, G., Bavencoffe, A., Lemonnier, L., Thebault, S., Shuba, Y., Barritt, G., Skryma, R. and Prevarskaya, N. Prospects for prostate cancer imaging and therapy using high-affinity TRPM8 activators. *Cell Calcium* 41 (2007) 285-294.
- [5] Rosado, J.A., Redondo, P.C., Pariente, J.A. and Salido, G. M. Calcium signalling and tumorigenesis. *Cancer Therapy* 2 (2004) 263-70.
- [6] Ramsey, S.I., Delling, M. and Clapham, D.E. An introduction to TRP channels. *Annu. Rev. Physiol.* 68 (2006) 18.1-18.29.
- [7] Nilius, B., Owsianik, G., Voets, T. and Peters, J.A. Transient receptor cation channels in disease. *Physiol. Rev.* 87 (2007) 165-217.
- [8] Duncan, L.M., Deeds, J., Hunter, J., Shao, J., Holmgren, L.M., Woolf, E.A., Tepper, R.I. and Shyjan, A.W. Down regulation of the novel gene melastatin correlates with potential for melanoma metastasis. *Cancer Res.* 58 (1998) 1515-20.
- [9] Fang, D. and Setaluri, V. Expression and up-regulation of alternatively spliced transcripts of melastatin, a melanoma metastasis-related gene, in human melanoma cells. *Biochem. Biophys. Res. Commun.* 279 (2000) 53-61.
- [10] Deeds, J., Cronin, F and Duncan, L.M. Patterns of melastatin mRNA expression in melanocytic tumors. *Hum. Pathol.* 31 (2000) 1346-56.
- [11] Duncan, L.M., Deeds, J., Cronin, F.E., Donovan, M., Sober, A.J., Kauffman, M. and McCarthy, J.J. Melastatin expression and prognosis in cutaneous malignant melanoma. *J. Clin. Oncol.* 19 (2001) 568-76.
- [12] Tsavaler, L., Shapero, M.H., Morkowski, S. and Laus, R. Trp-p8, a novel prostate-specific gene, is up-regulated in prostate cancer and other malignancies and shares high homology with transient receptor potential calcium channel proteins. *Cancer Res.* 61 (2001) 3760-9.
- [13] Fuessel, S., Sickert, D., Meye, A., Klenk, U., Schmidt, U., Schmitz, M. et al. Multiple tumour marker analyses (PSA, hK2, PSCA, trp-p8) in primary prostate cancers using quantitative RT-PCR. *Int. J. Oncol.* 23 (2003) 221-8.
- [14] Prevarskaya, N., Skryma, R., Bidaux, G. and Shuba, Y. Ion Channels in Death and Differentiation of Prostate Cancer Cells, *Cell Death and Differentiation* (2007) (in press).

- [15] Mergler, S., Strowski, M., Kaiser, S., Plath, T., Giesecke, Y., Neumann, M., Hosokawa, H., Kobayashi, S., Langrehr, J., Neuhaus, P., Plockinger, U., Wiedenmann, B., Grotzinger, C. Transient receptor potential channel TRPM8 agonists stimulate calcium influx and neurotensin secretion in neuroendocrine tumour cells. *Neuroendocrinology* (2007) Epub Apr5 in press.
- [16] Domotor, A., Peidl, Z., Vincze, A., Hunyady, B., Szolcsanyi, J., Kereskay, L., Szekeres, G. and Mozsik, G. Immunohistochemical distribution of vanilloid receptor, calcitonin-gene related peptide and substance P in gastrointestinal mucosa of patients with different gastrointestinal disorders. *Inflammopharmacology*. 13 (2005) 161-77.
- [17] Hartel, M., di Mola, F.F., Selvaggi, F., Mascetta, G., Wentz, M.N., Felix, K., Giese, N.A. Hinz, U., Di Sebastiano, P., Buchler, M.W. and Friess, H. Vanilloids in pancreatic cancer: potential for chemotherapy and pain management. *Gut*. 55 (2006) 519-28.
- [18] Lazzeri, M., Vannucchi, M.G., Spinelli, M., Bizzoco, E., Beneforti, P., Turini, D. and Fausone-Pellegrini, M.S. Transient receptor potential vanilloid type 1 (TRPV1) expression changes from normal urothelium to transitional cell carcinoma in human bladder. *Eur. Urol.* 48 (2005) 691-8.
- [19] Sanchez, M.G., Sanchez, A.M., Collado, B., Malagarie-Cazenave, S., Olea, N., Carmena, M.J., Prieto, J.C. and Diaz-Laviada, I.I. Expression of the transient receptor potential vanilloid 1 (TRPV1) in LNCaP and PC-3 prostate cancer cells and in human prostate tissue. *Eur. J. Pharmacol.* 515 (2005) 20-27.
- [20] Fixemer, T., Wissenbach, U., Flockerzi, V. and Bonkhoff, H. Expression of the Ca²⁺-selective cation channel TRPV6 in human prostate cancer: a novel prognostic marker for tumour progression. *Oncogene* 22 (2003) 7858-61.
- [21] Zhuang, L., Peng, J.B., Tou, L., Takanaga, H., Adam, R.M., Hediger, M.A. and Freeman, M.R. Calcium-selective ion channel, CaT1, is apically localized in gastrointestinal tract epithelia and is aberrantly expressed in human malignancies. *Lab. Invest.* 82 (2002) 1755-64.
- [22] Wissenbach, U., Niemeyer, B., Himmerkus, N., Fixemer, T., Bonkhoff, H. and Flockerzi, V. TRPV6 and prostate cancer: cancer growth beyond the prostate correlates with increased TRPV6 Ca²⁺ channel expression. *Biochem Biophys Res Commun* 322 (2004) 1359-63.
- [23] Peng, J.B., Zhuang, L., Berger, U.V., Adam, R.M., Williams, B.J., Brown, E.M., Hediger, M.A. and Freeman, M.R. CaT1 expression correlates with tumour grade in prostate cancer. *Biochem. Biophys. Res. Commun.* 282 (2001) 729-34.
- [24] Peng, J.B., Chen, X.Z., Berger, U.V., Weremowicz, S., Morton, C.C., Vassilev, P.M. and Brown, E.M. Human calcium transport protein CaT1. *Biochem. Biophys. Res. Commun.* 278 (2000) 326-32.
- [25] Bodding, M. TRP proteins and cancer. *Cellular Signalling* 19 (2007) 617-624.
- [26] Menendez, L., Juarez, L., Garcia, E., Garcia-Suarez, O., Hidalgo, A. and Baamonde, A. Analgesic effects of capsazepine and resiniferatoxin on bone cancer pain in mice. *Neurosci. Lett.* 393 (2006) 70-73.

- [27] Ghilardi, J.R., Rohrich, H., Lindsay, T.H., Sevcik, M.A., Schwei, M.J., Kubota, K., Halvorson, K.G., Poblete, J., Chaplan, S.R., Dubin, A.E., Carruthers, N.I., Swanson, D., Kuskowski, M., Flores, C.M., Julius, D. and Mantyh, P.W. Selective blockade of the capsaicin receptor TRPV1 attenuates bone cancer pain. *J. Neurosci.* 25 (2005) 3126-31.
- [28] McKemy, D. D., Neuhauser, W.M. and Julius, D. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature* 416 (2002) 52-8.
- [29] Peier, A.M., Moqrich, A., Hergarden, A.C., Reeve, A.J., Andersson, D.A., Story, G.M. et al. A TRP channel that senses cold stimuli and menthol. *Cell* 108 (2002) 705-15.
- [30] Voets, T., Droogmans, G., Wissenbach, U., Janssens, A., Flockerzi, V. and Nilius, B. The principle of temperature-dependent gating in cold- and heat-sensitive TRP channels. *Nature* 430 (2004) 748-754.
- [31] Liu, B., and Qin, F. J. Functional control of cold- and menthol-sensitive TRPM8 ion channels by phosphatidylinositol 4,5-bisphosphate. *Neurosci.* 25 (2005) 1674-1681.
- [32] Rohacs, T., Lopes, C.M., Michailidis, I. and Logothetis, D.E. PI (4,5) P2 regulates the activation and desensitization of TRPM8 channels through the TRP domain. *Nat. Neurosci.* 8 (2005) 626-634.
- [33] Vanden Abeele, F., Zholos, A., Bidaux, G., Shuba, Y., Thebault, S., Beck, B., Flourakis, M., Panchin, Y., Skryma, R. and Prevarskaya, N. Ca^{2+} -independent phospholipase A(2)-dependent gating of TRPM8 by lysophospholipids. *J. Biol. Chem.* 281 (2006) 40174-82.
- [34] Andersson, D., Nash, M. and Bevan S. Modulation of the cold-activated channel TRPM8 by lysophospholipids and polyunsaturated fatty acids. *J. Neuroscience*, 27 (2007) 3347-55.
- [35] Zhang, L. and Barritt, G.J. Evidence that TRPM8 is an androgen-dependent Ca^{2+} channel required for the survival of prostate cancer cells. *Cancer Res.* 64 (2004) 8365-73.
- [36] Tsuzuki, K., Xing, H., Ling, J. and Gu, J.G. Menthol-induced Ca^{2+} release from presynaptic Ca^{2+} stores potentiates sensory synaptic transmission. *J. Neurosci.* 24 (2004) 762-771.
- [37] Thebault, S., Lemonnier, L., Bidaux, G., Flourakis, M., Bavencoffe, A., Gordienko, D et al. Novel role of cold/menthol-sensitive transient receptor potential melastatine family member 8 (TRPM8) in the activation of store-operated channels in LNCaP human prostate cancer epithelial cells. *J. Biol. Chem.* 280 (2005) 39423-35.
- [38] Wolf, M.J., Wang, J., Turk, J. and Gross, R.W. Depletion of intracellular calcium stores activates smooth muscle cell calcium-independent phospholipase A2. A novel mechanism underlying arachidonic acid mobilization. *J. Biol. Chem.* 272 (1997) 1522-1526.

- [39] Stein, R.J., Santos, S., Nagatomi, J., Hayashi, Y., Minnery, B.S., Xavier, M., Patel, A.S., Nelson, J.B., Futrell, W.J., Yoshimura, N., et al. Cool (TRPM8) and hot (TRPV1) receptors in the bladder and male genital tract. *J. Urol.* 172 (2004) 1175-1178.
- [40] Zhang, L. and Barritt, G.J. TRPM8 in prostate cancer cells: a potential diagnostic and prognostic marker with a secretory function? *Endocr. Relat. Cancer* 13 (2006) 27-38.
- [41] Bidaux, G., Roudbaraki, M., Merle, C., Crepin, A., Delcourt, P., Slomianny, C et al. Evidence for specific TRPM8 expression in human prostate secretory epithelial cells: functional androgen receptor requirement. *Endocr. Relat. Cancer* 12 (2005) 367-82.
- [42] Bonkhoff, H., Fixemer, T. and Remberger, K. Relation between Bcl-2, cell proliferation, and the androgen receptor status in prostate tissue and precursors of prostate cancer. *Prostate* 34 (1998) 251-8.
- [43] Bruckheimer, E.M., Spurgers, K., Weigel, N.L., Logothetis, C. and McDonnell, T.J. Regulation of Bcl-2 expression by dihydrotestosterone in hormone sensitive LNCaP-FGC prostate cancer cells. *J. Urol.* 169 (2003) 1553-7.
- [44] Nantermet, P.V., Xu, J., Yu, Y., Hodor, P., Holder, D., Adamski, S et al. Identification of genetic pathways activated by the androgen receptor during the induction of proliferation in the ventral prostate gland. *J. Biol. Chem.* 279 (2004) 1310-22.
- [45] Kiessling, A., Fussel, S., Schmitz, M., Stevanovic, S., Meye, A., Weigle, B. et al. Identification of an HLA-A*0201-restricted T-cell epitope derived from the prostate cancer-associated protein trp-p8. *Prostate* 56 (2003) 270-9.
- [46] Berges, R.R., Vukanovic, J., Epstein, J.I., CarMichel, M., Cisek, L., Johnson, D.E., Veltri, R.W., Walsh, P.C. and Isaacs, J.T. Implication of cell kinetic changes during the progression of human prostatic cancer. *Clin. Cancer Res.* 1 (1995) 473-80.
- [47] Henshall, S.M., Afar, D.E., Hiller, J., Horvath, L.G., Quinn, D.I., Rasiyah, K.K. et al. Survival analysis of genome-wide gene expression profiles of prostate cancers identifies new prognostic targets of disease relapse. *Cancer Res.* 63 (2003) 4196-203.
- [48] Bidaux, G., Flourakis, M., Thebault, S., Zholos, A., Beck, B., Gkika, D., Roudbaraki, M., Bonnal, J.L., Mauroy, B., Shuba, Y., Skryma, R. and Prevarskaya, N. Differentiation status of prostate cells determines TRPM8 channel subcellular localization and function: involvement in carcinogenesis. *J. Clin. Invest.* (2007) (in press).
- [49] Legrand, G., Humez, S., Slomianny, C., Dewailly, E., Vanden Abeele, F., Mariot, P., Wuytack, F. and Prevarskaya, N. Ca^{2+} pools and cell growth. Evidence for sarcoendoplasmic Ca^{2+} -ATPases 2B involvement in human prostate cancer cell growth control. *J. Biol. Chem.* 276 (2001) 47608-47614.
- [50] Thebault, S., Roudbaraki, M., Sydorenko, V., Shuba, Y., Lemonnier, L., Slomianny, C., Dewailly, E., Bonnal, J.L., Mauroy, B., Skryma, R., et al. Alpha1-adrenergic receptors activate Ca^{2+} -permeable cationic channels in prostate cancer epithelial cells. *J. Clin. Invest.* 111 (2003) 1691-1701.
- [51] Thebault, S., Flourakis, M., Vanoverberghe, K., Vandermoere, F., Roudbaraki, M., Lehen'kyi, V., Slomianny, C., Beck, B., Mariot, P., Bonnal, J.L., et al. Differential role

- of transient receptor potential channels in Ca^{2+} entry and proliferation of prostate cancer epithelial cells. *Cancer Res.* 66 (2006) 2038-2047.
- [52] Vanoverberghe, K., Vanden Abeele, F., Mariot, P., Lepage, G., Roudbaraki, M., Bonnal, J.L., Mauroy, B., Shuba, Y., Skryma, R. and Prevarskaya, N. Ca^{2+} homeostasis and apoptotic resistance of neuroendocrine-differentiated prostate cancer cells. *Cell Death Differ.* 11 (2004) 321-330.
- [53] Skryma, R., Mariot, P., Bourhis, X.L., Coppenolle, F.V., Shuba, Y., Vanden Abeele, F., Legrand, G., Humez, S., Boilly, B. and Prevarskaya, N. Store depletion and store-operated Ca^{2+} current in human prostate cancer LNCaP cells: involvement in apoptosis. *J. Physiol.* 527 (2000) 71-83.
- [54] Vanden Abeele, F., Skryma, R., Shuba, Y., Van Coppenolle, F., Slomianny, C., Roudbaraki, M., Mauroy, B., Wuytack, F. and Prevarskaya, N. Bcl-2-dependent modulation of Ca^{2+} homeostasis and store-operated channels in prostate cancer cells. *Cancer Cell* 1 (2002) 169-179.
- [55] Xu, X.Z., Moebius, F., Gill, D.L. and Montell, C. Regulation of melastatin, a TRP-related protein, through interaction with a cytoplasmic isoform. *Proc. Nat. Acad. Sci. USA* 98 (2001) 19692-7.
- [56] Lis, A., Wissenbach, S.E. and Philipp, S.E. Transcriptional regulation and processing increase the functional variability of TRPM channels. *Naunyn-Schmiedebergs Arch. Pharmacol.* 371 (2005) 315-24.
- [57] Miller, A.J., Du, J., Rowan, S., Hershey, C.L., Widlund, H.R. and Fisher, D.E. Transcriptional regulation of the melanoma prognostic marker melastatin (TRPM1) by MITF in melanocytes and melanoma. *Cancer Res.* 64 (2004) 509-16.
- [58] Zhiqi, S., Soltani, M.H., Bhat, K.M., Sangha, N., Fang, D., Hunter, J.J. and Setaluri, V. Human melastatin 1 (TRPM1) is regulated by MITF and produces multiple polypeptide isoforms in melanocytes and melanoma. *Melanoma Res.* 14 (2004) 509-16.
- [59] Hunter, J.J., Shao, J., Smutko, J.S., Dussault, B.J., Nagle, D.L., Woolf, E.A., Holmgren, L.M., Moore, K.J. and Shyjan, A.W. Chromosomal localization and genomic characterization of the mouse melastatin gene (M1sn1). *Genomics* 54 (1998) 116-23.
- [60] Yue, L., Peng, J.B., Hediger, M. and Clapham, D.E. CaT1 manifests the pore properties of the calcium-release-activated calcium channel. *Nature* 410 (2001) 704-9.
- [61] Bodding, M., Wissenbach, U. and Flockerzi, V. The recombinant human TRPV6 channel functions as Ca^{2+} sensor in human embryonic kidney and rat basophilic leukemia cells. *J. Biol. Chem.* 277 (2002) 36656-64.
- [62] Bodding, M. and Flockerzi, V. Ca^{2+} dependence of the Ca^{2+} -selective TRPV6 channel. *J. Biol. Chem.* 279 (2004) 36546-52.

- [63] Schwarz, E.C., Wissenbach, U., Niemeyer, B.A., Strauss, B., Philipp, S.E., Flockerzi, V. and Hoth, M. TRPV6 potentiates calcium-dependent cell proliferation. *Cell Calcium* 39 (2006) 163-73.
- [64] Wissenbach, U., Niemeyer, B.A., Fixemer, T., Schniedewind, A., Trost, C., Cavalie, A., Reus, K., Meese, E., Bonkhoff, H. and Flockerzi, V. Expression of CaT-like, a novel calcium-selective channel, correlates with the malignancy of prostate cancer. *J. Biol. Chem.* 276 (2001) 19461-8.
- [65] Peng, J.B., Chen, X.Z., Berger, U.V., Vassilev, P.M., Tsukaguchi, H., Brown, E.M. and Hediger, M.A. Molecular cloning and characterization of a channel-like transporter mediating intestinal calcium absorption. *J. Biol. Chem.* 274 (1999) 22739-22746.
- [66] Bianco, S.D., Peng, J.B., Takanaga, H., Suzuki, Y., Crescenzi, A., Kos, C.H., Zhuang, L., Freeman, M.R., Gouveia, C.H., Wu, J., Luo, H., Mauro, T., Brown, E.M. and Hediger, M.A. Marked disturbance of calcium homeostasis in mice with targeted disruption of the TRPV6 calcium channel gene. *J. Bone Miner Res.* 22 (2007) 274-85.
- [67] Taparia, S., Fleet, J.C., Peng, J.B., Wang, X.D. and Wood, R.J. 1,25-Dihydroxyvitamin D and 25-hydroxyvitamin D-mediated regulation of TRPV6 (a putative epithelial calcium channel) mRNA expression in Caco-2 cells. *Eur. J. Nutr.* 45 (2006) 196-204.
- [68] Lehen'kyi, V., Flourakis, M., Skryma, R. and Prevarskaya, N. TRPV6 channel controls prostate cancer cell proliferation via Ca^{2+} /NFAT-dependent pathways. *Oncogene* (2007) in press.
- [69] Vanden Abeele, F., Shuba, Y., Roudbaraki, M., Lemonnier, L., Vanoverberghe, K., Mariot, P., Skryma, R. and Prevarskaya, N. Store-operated Ca^{2+} channels in prostate cancer epithelial cells: function, regulation, and role in carcinogenesis. *Cell Calcium* 33 (2003) 357-73.
- [70] Bodding, M., Fecher-Trost, C. and Flockerzi, V. Store-operated Ca^{2+} current and TRPV6 channels in lymph node prostate cancer cells. *J. Biol. Chem.* 278 (2003) 50872-9.
- [71] Kahr, H., Schindl, R., Fritsch, R., Heinze, B., Hofbauer, M., Hack, M.E., Mortelmaier, M.A., Groschner, K., Peng, J.B., Takanaga, H., Hediger, M.A. and Romanin, C. CaT1 knock-down strategies fail to affect CRAC channels in mucosal-type mast cells. *J. Physiol.* 557 (2004) 121-32.
- [72] Vanden Abeele, F., Lemonnier, L., Thebault, S., Lepage, G., Parys, J.B., Shuba, Y., Skryma, R. and Prevarskaya, N. Two types of store-operated Ca^{2+} channels with different activation modes and molecular origin in LNCaP human prostate cancer epithelial cells. *J. Biol. Chem.* 279 (2004) 30326-37.
- [73] Vanden Abeele, F., Roudbaraki, F., Shuba, M., Skryma, Y. and Prevarskaya, N. Store-operated Ca^{2+} current in prostate cancer epithelial cells- Role of endogenous Ca^{2+} transporter type 1. *J. Biol. Chem.* 278 (2003) 15381-89.

- [74] Sydorenko, V., Shuba, Y., Thebault, S., Roudbaraki, M., Lepage, G., Prevarskaya, N. and Skryma, R. Receptor-coupled, DAG-gated Ca^{2+} -permeable cationic channels in LNCaP human prostate cancer epithelial cells. *J. Physiol.* 548 (2003) 823-36.
- [75] Suguro, M., Tagawa, H., Kagami, Y., Okamoto, M., Ohshima, K., Shiku, H., Morishima, Y., Nakamura, S. and Seto, M. Expression profiling analysis of the CD5(+) diffuse large B-cell lymphoma subgroup: Development of a CD5 signature. *Cancer Sci.* 97 (2006) 868-74.
- [76] Prawitt, D., Enklaar, T., Klemm, G., Gartner, B., Spangenberg, C., Winterpacht, A., Higgins, M., Pelletier, J. and Zabel, B. Identification and characterization of mTR1, a novel gene with homology to melastatic (MLSN1) and the TRP gene family located in the BWS-WT2 critical region on chromosome 11p15.5 and showing allele-specific expression. *Hum. Mol. Genet.* 9 (2000) 203-16.
- [77] Ahern, G.P., Wang, X. and Miyares, R.L. Polyamines are potent ligands for the capsaicin receptor TRPV1. *J. Biol. Chem.* 281 (2006) 8991-5.
- [78] Wertz, I.E. and Dixit, V.M. Characterization of calcium release-activated apoptosis of LNCaP prostate cancer cells. *J. Biol. Chem.* 275 (2000) 11470-8.
- [79] Tapia-Vieyra, J.V. and Mas-Oliva, J. Apoptosis and cell death channels in prostate cancer. *Archives of Medical Research* 32 (2001) 175-185.
- [80] Costa-Pereira, A.P. and Cotter, T.G. Molecular and cellular biology of prostate cancer – the role of apoptosis as a target for therapy. *Prostate Cancer and Prostatic Diseases* 2 (1999) 126-139.
- [81] Xue, W., Zender, L., Miething, C., Dickins, R.A., Hernando, E., Krizhanovsky, V., Cordon-Cardo, C. and Lowe, S.W. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature* 445 (2007) 656-60.
- [82] MacDiamid, J.A., Mugridge, N.B., Weiss, J.C., Phillips, L., Burn, A.L., Paulin, R.P., Haasdyk, J.E., Dickson, K-A., Brahmabhatt, V. N., Pattison, S.T., James, A.C., Bakri, G.A., Straw, R.C., Stillman, B., Graham, R.M. and Brahmabhatt, H. Bacterially derived 400 nm particules for encapsulation and cancer cell targeting of chemotherapeutics. *Cancer Cell* 11 (2007) 431-445.
- [83] Aarts, M., Iihara, K., Wei, W.L., Xiong, Z.G., Arundine, M., Cerwinski, W., MacDonald, J.F. and Tymianski, M. A key role for TRPM7 channel in anoxic neuronal death. *Cell* 115 (2003) 863-877.
- [84] McNulty, S. and Fonfria, E. The role of TRPM channels in cell death. *Eur. J. Physiology* 451 (2005) 235-42.
- [85] Orrenius, S., Gogvadze, V. and Zhivotovsky, B. Mitochondrial oxidative stress: implications for cell death. *Annu. Rev. Pharmacol. Toxicol.* 47 (2007) 143-83.
- [86] Lin, H., Juang, J.L. and Wang, P.S. Involvement of Cdk5/p25 in digoxin-triggered prostate cancer cell apoptosis. *J. Biol. Chem.* 279 (2004) 29302-07.

- [87] Zhang, L., Brereton, H.M., Hahn, M., Froscio, M., Tilley, W.D., Brown, M.P. and Barritt, G.J. Expression of *Drosophila* Ca²⁺ permeable transient receptor potential-like channel protein in a prostate cancer cell line decreases cell survival. *Cancer Gene Therapy* 10 (2003) 611-625.
- [88] Horomito, M., Sasaki, Y., Ugawa, S. et al. A novel strategy for cancer therapy by mutated mammalian degenerin gene transfer. *Cancer Gene Therapy* 7 (2000) 1341-1347.
- [89] Ruengrairatanaroje, P., Hahn, M., Brereton, H.M., Zhang, L., Froscio, M., Petronijevic, T. and Barritt, G.J. Development of a tetracycline-inducible system for expression of the Ca²⁺ permeable TRPL channel and the killing of prostate cancer cells. *Cancer Therapy* 2 (2004) 389-402.
- [90] Denmeade, S.R. and Isaacs, J.T. The SERCA pump as a therapeutic target. Making a 'smart bomb' for prostate cancer. *Cancer Biology and Therapy* 4 (2005) 14-22.
- [91] Lan, L., Bawden, M.J., Auld, A.M. and Barritt, G.J. Expression of *Drosophila* trp1 cRNA in *Xenopus laevis* oocytes leads to the appearance of a Ca²⁺ channel activated by Ca²⁺ and calmodulin, and by guanosine 5' [gamma-thio]triphosphate. *Biochem J.* 316 (1996) 793-803.
- [92] Gillo, B., Chorna, I., Cohen, H., Cook, B., Manistersky, I., Chorev, M., Arnon, A., Pollock, J.A., Sellinger, Z., Minke, B. Coexpression of *Drosophila* TRP and TRP-like proteins in *Xenopus* oocytes reconstitutes capacitative Ca²⁺ entry. *Proc. Nat. Acad. Sci. USA.* 93 (1996) 14146-51.
- [93] Zimmer, S., Trost, C., Wissenbach, U., Phillipp, S., Freichel, M., Flockerzi, V. and Cavalle, A. Modulation of recombinant transient-receptor-potential-like (TRPL) channels by cytosolic Ca²⁺. *Pflugers Arch. Eur. J. Physiology* 440 (2000) 409-417.
- [94] Reilly, C.A., Taylor, J.L., Lanza, D.L., Carr, B.A., Crouch, D.J. and Yost, G.S. Capsaicinoids cause inflammation and epithelial cell death through activation of vanilloid receptors. *Toxicol Sci.* 73 (2003) 170-81.
- [95] DeFeo-Jones, D., Garsky, V.M., Wong, B.K., Feng, D.M. Bolyar, T., Haskell, K., Kiefer, D.M., Leander, K., McAvoy, E., Lumma, P., Wai, J., Senderak, E.T., Motzel, S.L., Keenan, K., Van Zwieten, M., Lin, J.H., Freidinger, R., Huff, J., Oliff, A. and Jones, R.E. A peptide-doxorubicin 'prodrug' activated by prostate-specific antigen selectively kills prostate tumor cells positive for prostate-specific antigen *in vivo*. *Nature Medicine* 6 (2000) 1248-1252.
- [96] Garsky, V.M., Lumma, P.K., Feng, D.M., Wai, J., Ramjit, H.G., Sardana, M.K., Oliff, A., Jones, R.E., DeFeo-Jones, D. and Freidinger, R.M. The synthesis of a prodrug of doxorubicin designed to provide reduced systemic toxicity and greater target efficacy. *J. Med. Chem.* 44 (2001) 4216-24.
- [97] Ligresti, A., Moriello, A.S., Starowicz, K., Matias, I., Pisanti, S., De Petrocellis, L., Laezza, C., Portella, G., Bifulco, M. and Di Marzo, V. Antitumour activity of plant cannabinoids with emphasis on the effect of cannabidiol on human breast carcinoma. *J. Pharmacol. Exp. Ther.* 318 (2006) 1375-87.

- [98] Izzo, A.A. and Coutts, A.A. Cannabinoids and the digestive tract. *Handb. Exp. Pharmacol.* 168 (2005) 573-98.
- [99] Asai, H., Ozaki, N., Shinoda, M., Nagamine, K., Tohnai, I., Ueda, M. and Sugiura, Y. Heat and mechanical hyperalgesia in mice model of cancer pain. *Pain.* 117 (2005) 19-29.

ACCEPTED MANUSCRIPT

Legends to Figures

Fig. 1. Development of strategies to kill cancer cells using sustained Ca^{2+} and Na^+ entry through plasma membrane TRP channels. (A) A scheme showing pathways of Ca^{2+} - and Na^+ -induced cell death resulting from sustained Ca^{2+} and Na^+ entry, including the concentrations of Ca^{2+} in mitochondria ($\text{Ca}^{2+}_{\text{M}}$) and ER ($\text{Ca}^{2+}_{\text{ER}}$). (B) Heterologous expression of TRPV1 in LNCaP prostate cancer cells under the control of the CMV promoter. TRPV1 was cloned from DNA from nodose ganglia, inserted into the Ad-Track plasmid (which can express EGFP), and transiently transfected into LNCaP prostate cancer cells. (a) Western Blot showing expression of endogenous TRPV1 in extracts of dorsal root ganglia, and in LNCaP cells transfected with Ad-Track-CMV-TRPV1. (b) Images showing localisation of TRPV1 by immunofluorescence (panels 1 and 2) and GFP fluorescence (panels 3 and 4) (Zhang, R. and Barritt, G. unpublished results). (C) The activation of TRPV1 channels heterologously expressed in LNCaP cells with capsaicin leads to a substantial decrease in cell numbers (detected by GFP fluorescence) (Zhang, R. and Barritt, G. unpublished results). (D) A representation of the structure of a pro-drug [96] in which capsaicin is covalently linked to a prostate specific antigen (PSA)-cleavable peptide (Zhang, R., Voelker, N. and Barritt, G.J. unpublished results). The serine protease activity of PSA would be expected to hydrolyse the Gln-Ser bond to yield Ser-Leu-capsaicin which can potentially bind to the TRPV1 ligand site.

Table 1. TRP Proteins Exhibiting Altered Expression in Cancer Cells.

TRP Protein	Likely Physiological Function in Normal Cells	Cancers Investigated	Changes in TRP Expression	References
TRPM1	Not determined.	Melanoma	Decreased expression in tumorigenic melanocytes	[8-11]
TRPM8	Sensor for noxious cold, and pain.	Prostate Pancreas	Increased expression in androgen-insensitive prostate cancer cells	[12-15]
TRPV1	Sensor for noxious heat, acid.	Prostate Bladder Colon Pancreas	Increased expression in prostate cancer, colon cancer and pancreatic cancer. Decreased expression as bladder cancer progresses and cells de-differentiate.	[16-19] [18]
TRPV6	Ca ²⁺ across intestinal epithelial cells.	Prostate Breast Thyroid Colon Ovarian	Increased expression in cancer cells.	[20-24]

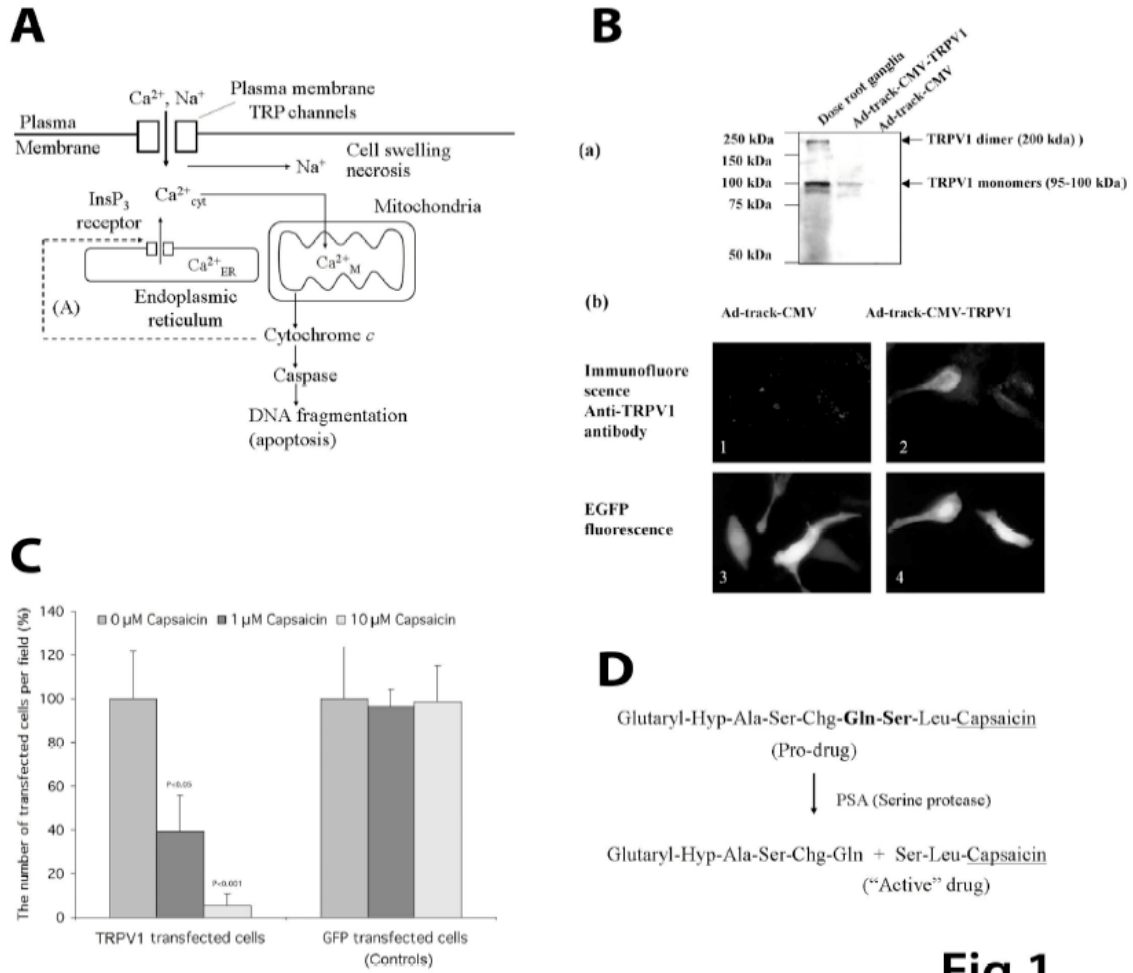


Fig 1

ACC