Differential role of TRP channels in prostate cancer

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Abstract

A major clinical problem with PC (prostate cancer) is the cell's ability to survive and proliferate upon androgen withdrawal. Indeed, deregulated cell differentiation and proliferation, together with the suppression of apoptosis, provides the condition for abnormal tissue growth. Here, we examine the differential role of TRP (transient receptor potential) channels in the control of Ca^{2+} homoeostasis and growth of PC cells.

Introduction

PC (prostate cancer) is one of the leading threats to men's health. Its early stage depends on androgens for growth and survival, and androgen ablation therapy may at this time be effective in causing tumours to regress; however, in the late androgen-independent stage there is currently no successful therapy. It is, therefore, vital to understand what drives the progression to androgen independence. The latter is associated with the appearance of new cell phenotypes, characterized by apoptosis inhibition and aberrant cell proliferation. Deregulated cell differentiation and proliferation together with the suppression of apoptosis provides the conditions for abnormal tissue growth, which ultimately can turn into uncontrolled expansion and invasion characteristics of cancer.

The role of calcium (Ca^{2+}) in the overall cancer-related cell signalling pathways is uncontested. Alterations in Ca^{2+} homoeostasis have been described to increase proliferation [1,2] and to induce differentiation [3] or apoptosis [4–6]. According to a growing number of studies, cationic channels in the TRP (transient receptor potential) family are key players of calcium homoeostasis and cell physiopathology. In the last few years, it has emerged that several members of the TRP family could play an important role in prostate carcinogenesis and, moreover, some of them have been suggested as prognostic markers for PC, with particular use in differential diagnosis [6].

Here, we examine the differential role of TRP channels in Ca^{2+} homoeostasis of PC epithelial cells and their respective role in prostate carcinogenesis. Recent progress achieved in our understanding of molecular mechanisms of TRP channel signalling involved in the control of PC progression ensures that sooner or later fundamental breakthroughs will progress to practical applications.

Agonist-dependent growth regulation of human PC epithelial cells: the role of TRPC (TRP canonical) channels

Various growth factors, neurotransmitters and hormones, known to control physiological and pathological cell proliferation, participate in the maintenance of intracellular Ca^{2+} homoeostasis. Although the nature of these agonists has yet to be well established during PC progression, they invariably induce a Ca^{2+} entry called agonist-induced Ca^{2+} entry. Using the androgen-dependent LNCaP (lymph node carcinoma of the prostate) cell line and primary cultures of human PC epithelial cells established from resection specimens, we have demonstrated that α_1-AR (α_1-adrenergic receptor) stimulation activates non-specific cationic channels, leading to agonist-induced Ca^{2+} entry [7].

Interestingly, we have also shown that in contrast with the stimulatory role of α_1-ARs in PC cell growth, metabotropic purinergic receptors (P2Y-R) are involved in the growth arrest of human PC cells [8]. Such divergent effects of two receptors on cell proliferation are surprising, since both α_1-AR and P2Y-R are known to be coupled with the common phospholipase C-catalysed inositol phospholipids breakdown signalling pathway, via which α_1-agonists and extracellular ATP could apparently induce similar increases in $[Ca^{2+}]_i$ (intracellular free Ca^{2+}). The opposite effects on cell proliferation can only be explained if the agonist-induced Ca^{2+} entry controlled by each receptor utilizes different Ca^{2+}-permeable membrane channels ultimately destined to target various intracellular effectors. We have shown that the pattern of Ca^{2+} signalling initiated by α_1-AR stimulation is characterized by regular oscillatory activity, which is almost exclusively based on the Ca^{2+} entry pathway directly gated by DAG (diacylglycerol) with no apparent role for IP_3 (inositol trisphosphate)-mediated store depletion. In contrast, Ca^{2+} signalling coupled with P2Y-R stimulation is largely determined by IP_3-mediated store-dependent processes,

Figure 1 | Schematic diagram showing in simplified form differential TRPM8 localization and function dependence on androgen receptor (AR) activity of human prostate epithelial cells and on their differentiation status
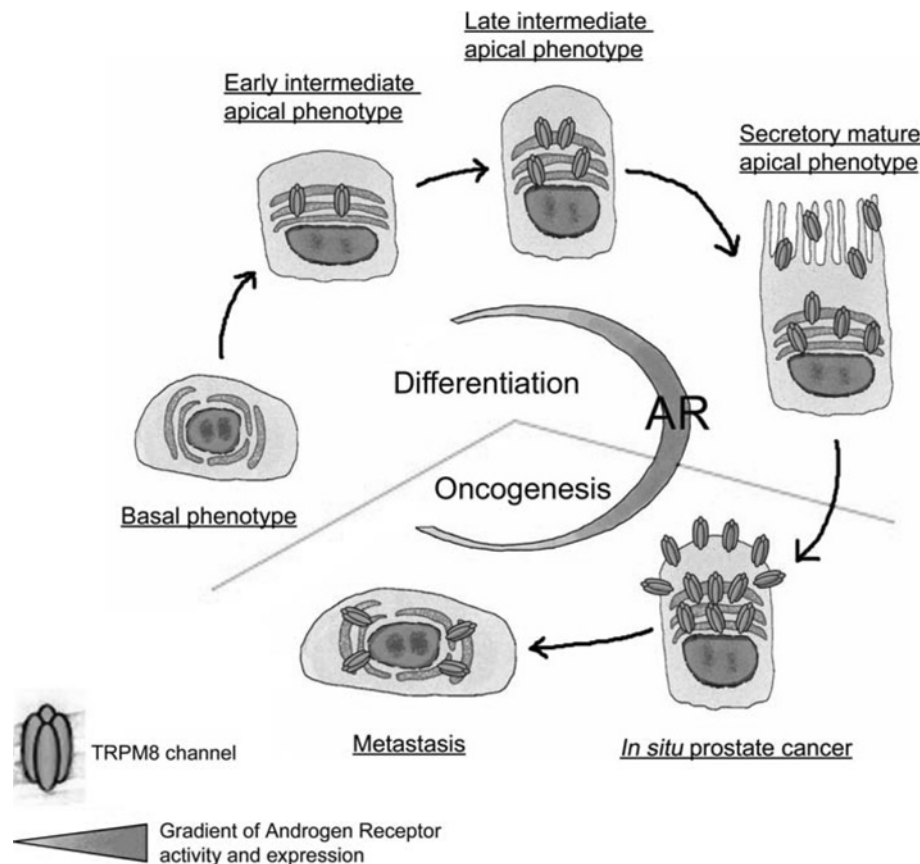
including robust Ca^{2+} release and the activation of store-operated Ca^{2+} influx.

Our results highlight the importance of Ca^{2+} entry pathways in the discrimination of the signalling via α_1-ARs and P2Y-purinergic receptors in hPCE cells. Indeed, the α_1-AR agonists activate Ca^{2+} entry mainly via the TRPC6 channel, whereas ATP-evoked Ca^{2+} entry predominantly involves TRPC1 and TRPC4 channels. TRPC6 silencing by antisense hybrid depletion or by siRNA (small interfering RNA) decreased both hPCE cell proliferation and agonist-induced Ca^{2+} entry. In contrast, agonist-induced Ca^{2+} entry and related growth arrest associated with P2Y-R stimulation involved neither TRPC6 nor NFAT (nuclear factor of activated T-cells). Thus the TRPC6 channel (which determines the oscillatory pattern of Ca^{2+} signalling coupling the agonist-mediated α_1-AR stimulation with Ca^{2+}-dependent activation of the NFAT transcription factor) could potentially represent a suitable target for therapeutic intervention.

TRPM8 (TRP melastatin 8) localization and function in prostate depends on the differentiation status: role in carcinogenesis

TRPM8 is a so-called 'cold' receptor belonging to the melastatin (TRPM) subfamily of TRP channels and is activated by cooling temperatures and menthol. Aside from sensory neurons, in which the role of TRPM8 in mediating cold-evoked excitation is fairly well established [9,10], this channel is most abundantly expressed in the prostate. In fact, TRPM8 was first cloned from the human prostate as a prostate-specific gene [11], even before its role in the cold sensation was established. Moreover, while remaining at moderate levels in normal prostate, TRPM8 expression strongly increases in PC. Despite the growing number of studies, the role of TRPM8 in prostate remains unclear, and it has been suggested to be involved in secretion function of the prostate and in the regulation of proliferation and/or apoptosis [12,13].

It has been also shown that anti-androgen therapy greatly reduced the expression of TRPM8, suggesting that TRPM8 is regulated by androgens [14]. We have demonstrated that in PC cell lines, and in primary cultures of normal, hyperplasic and cancerous prostate epithelial cells, TRPM8 is a target gene of the androgen receptor [12]. TRPM8 expression-silencing experiments using siRNA suggest that the Ca^{2+} inflow through TRPM8 plays an essential role in cellular Ca^{2+} homoeostasis in prostate epithelial cells and is involved in cell survival [13].

Our results [15] and those of Zhang and Barritt [13] indicate that TRPM8 may be expressed not just in the PM (plasma membrane), but also in the ER (endoplasmic reticulum)

membrane. Dual localization and channel-like function of TRPM8 in the two membranes significantly broaden the spectrum of physiological and pathological processes it may be involved in. However, the mechanisms that determine the preferred localization of TRPM8 in cells of different phenotypes are not known so far. Our results demonstrate that only highly differentiated human prostate primary luminal epithelial cells express functional PM [$_{PM}$TRPM8 (PM TRPM8)] channels. Moreover, prostate epithelium cancer cells (obtained from *in situ* PCs) were characterized by significantly larger $_{PM}$TRPM8-mediated current density than normal cells. We have shown that this $_{PM}$TRPM8 activity was abolished in dedifferentiated cells that had lost their luminal secretory phenotype. However, we found that, in contrast with $_{PM}$TRPM8, $_{ER}$TRPM8 (ER TRPM8) remained functional (as an ER Ca^{2+} release channel), independently of its differentiation status. Furthermore, similarly to dedifferentiated prostate epithelial cells, metastatic, LNCaP cells also exhibited most exclusive TRPM8 localization in the ER [15]. We hypothesize (Figure 1) that in prostate, TRPM8 localization may depend on epithelial cell phenotype (i.e. fully differentiated secretory apical cells versus non-differentiated basal cells) and on androgen status (i.e. androgen-dependent versus hormone refractory cells highly resistant to apoptosis). Taken together, these results suggest that TRPM8 may contribute to the initiation, promotion and progression of carcinogenesis in prostate epithelial cells.

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