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Canonical transient receptor potential channels in disease: targets for novel drug therapy?

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The canonical transient receptor potential (TRPC) channels constitute one of the three major families within the large transient receptor potential (TRP) superfamily. TRPC channels are the closest mammalian homologues of *Drosophila* TRP, the light-activated channel in *Drosophila* photoreceptor cells. All TRPC channels (TRPC1–7) are activated via phospholipase-C-coupled receptors and were, therefore, proposed to encode elusive native receptor-activated cation channels in many cell types. A physiological role has been established for all of the known TRPC channels, including the control of vascular tone (TRPC1, TRPC4 and TRPC6) or lymphocyte activation, which is essential for immune competence (TRPC1 and TRPC3). The emergence of TRPC channels in controlling a variety of biological functions offers new and promising targets for drug development.

Introduction

The elevation of intracellular free calcium (Ca^{2+}) represents one of the early events following stimulation of a variety of cell surface receptors and is fundamental for cell survival and function. This Ca^{2+} elevation can be multiphasic in nature, including contributions from release from intracellular stores, mainly the endoplasmic reticulum (ER), as well as influx across the plasma membrane. Ca^{2+} influx through plasma membrane channels is essential for neurotransmitter release at the synapse, muscle cell contraction, epithelial cell secretion, nitric oxide (NO) production by endothelial cells and successful lymphocyte activation in response to antigens [1]. Thus, dysfunction in Ca^{2+} homeostasis can result in diseases such as immunodeficiency, hypertension, neurodegenerative diseases and cancer [2–5].

Receptor-operated Ca^{2+} entry pathways

Stimulation of cells with agonists acting on Gq/11-coupled receptors or receptor tyrosine kinase activates the phosphoinositide (PI)-specific phospholipase C (PLC). PLC breaks down phosphatidylinositol(4,5)bisphosphate (PIP_2) to produce two second messengers, inositol(1,4,5)trisphosphate (IP_3) and diacylglycerol (DAG). A variety of Ca^{2+} entry routes with diverse activation mechanisms are triggered downstream of PLC activation. These Ca^{2+} entry pathways include those activated by second messengers generated from the PI pathway (i.e. IP_3, DAG, arachidonic acid) and therefore do not depend on the state of filling of the ER Ca^{2+} stores [6]. In addition, IP_3-mediated depletion of ER Ca^{2+} stores activates the ubiquitous store-operated Ca^{2+} (SOC) entry (originally termed capacitative Ca^{2+} entry or CCE [7]). The channels mediating SOC entry are called SOC channels, whereas all other channels activated downstream of PLC with processes not related to store depletion are referred to as non-SOC [7].

SOC channels have been the subject of numerous investigations in recent years [7]. SOC can be activated by every procedure that depletes the ER Ca^{2+} stores. This is best illustrated by the activation of SOC channels with thapsigargin, a specific inhibitor of the ER Ca^{2+} pump that causes passive depletion of the ER Ca^{2+} stores [7]. Current-mediating SOC entry was first characterized in hematopoetic cells and termed I_{CRAC} for Ca^{2+} release-activated Ca^{2+} current [7,8]. Although I_{CRAC}, a highly Ca^{2+}-selective current, remains the best characterized SOC current, other SOC currents with diverse electrophysiological characteristics have been identified in other cell types of non-hematopoetic origin [7]. A silencing RNA (RNAi)-based screen identified a Ca^{2+}-binding protein, stromal interaction molecule 1 (STIM1), as an essential player in the activation of SOC channels [9,10]. STIM1 is proposed to be the long sought sensor of Ca^{2+} content in the ER. Recently, two independent studies revealed that a membrane

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protein called Orai1/CRACM1 (CRAC channel modulator 1) is an essential component of I_{CRAC} [2,11].

Canonical transient receptor potential channels

Members of the canonical transient receptor potential (TRPC) family of channels have emerged as plausible candidates for native channels responsible for receptor-regulated Ca^{2+} entry [7]. Although recent data seem to suggest that Orai1/CRACM1 might be the pore-forming unit of the archetypical I_{CRAC} [12], it remains possible that TRPC proteins associate with Orai1/CRACM1 to form such channels [13,14]. At the very least, TRPC proteins remain candidates for other SOC channels of non-CRAC type, and more-convincing evidence points towards TRPC channels as components of non-SOC channels, such as second-messenger-activated channels [15].

The TRPC family belongs to the large transient receptor potential (TRP) superfamily [16–18]. The TRP superfamily now comprises >30 members and can be divided into seven families. The three major families are: 1) The vanilloid TRP (TRPV) channels, homologous to the vanilloid receptor, activated by a variety of signals including vanilloid compounds such as capsaicin, noxious signals, hypotonic cell swelling and heat [19]; 2) Melastatin-related TRP (TRPM) channels. They have diverse functional properties such as controlling Mg^{2+} entry, modulating the membrane potential, and sensing cold and menthol in sensory neurons [20]; 3) The TRPC family, containing seven members, which are activated through PLC-coupled receptors [21].

The structures of TRPC channels are shown in Figure 1. TRPC2 and TRPC3/6/7 were shown to be activated by DAG [22,23], whereas the exact mechanism by which PLC activates TRPC1/4/5 remains unknown. Figure 2 summarizes known TRPC domains that are important for TRPC regulation and interaction with other signaling molecules [21,24]. Given that all seven members of the TRPC family have in common activation through PLC-coupled receptors, they have been proposed to encode components of native receptor-operated channels (SOC and non-SOC) in different cell types [7]. In this review, I will focus on the members of the TRPC family of channels and their potential use as targets for drug therapy. For the involvement of the wider TRP superfamily in disease, see a recent article by Nilius and colleagues [25].

Canonical transient receptor potential channels as targets for drug therapy in human disease

Receptor-operated Ca^{2+} (ROC) channels, SOC and non-SOC, are widely expressed and can be found in non-excitable and electrically excitable cells [7]. However, with few exceptions, it remains difficult to match currents mediated by ectopically expressed TRP channels with those of ROC channels in their native environment. It is believed, by analogy with potassium channels, that functional TRPC channels are generated *in situ* by association of four TRPC proteins to form either homotetramers or heterotetramers [26]; additional accessory molecules might be involved [24]. The precise subunit composition, as well as the stoichiometry of each TRPC

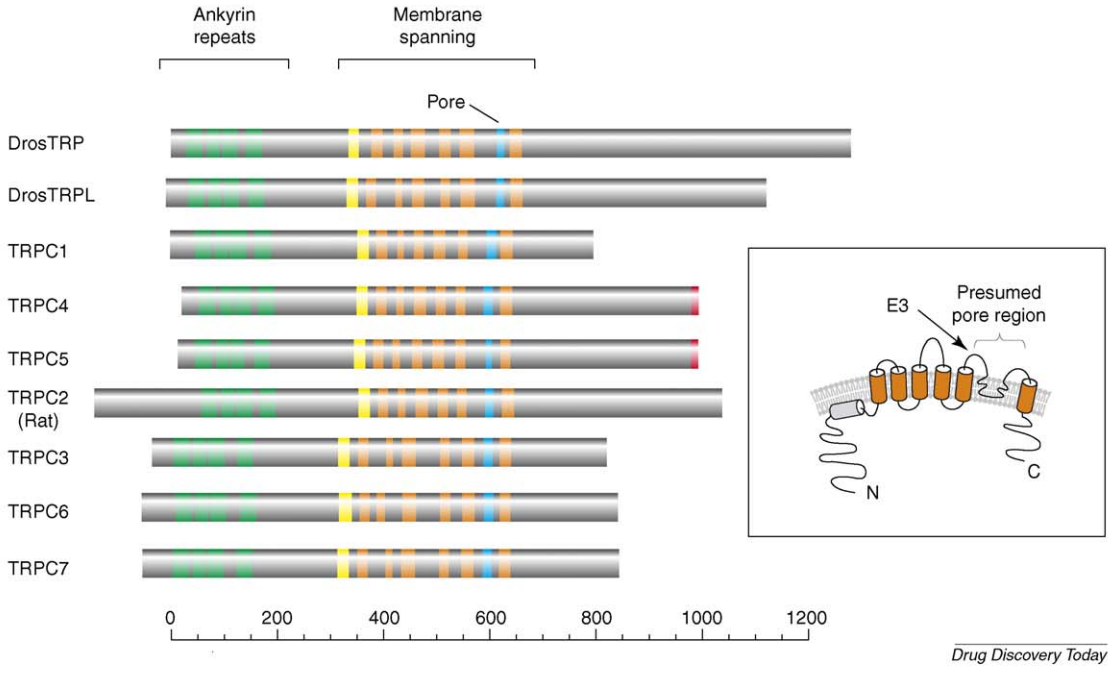

FIGURE 1

The canonical transient receptor potential family of cation channels. Structure of *Drosophila* transient receptor potential (TRP) and TRP-Like (TRPL) [17] and their mammalian homologues, canonical transient receptor potential (TRPC)1 though to 7, (TRPC2 is a pseudogene in humans; rat TRPC2 is depicted). All TRPC proteins are predicted to have cytoplasmic N- and C-termini, six membrane-spanning domains (orange) [in addition to a hydrophobic region that presumably does not span the membrane (yellow)], a predicted pore sequence (blue) and four ankyrin-like repeats in the cytoplasmic N-terminal region (green). TRPC4 and TRPC5 have an additional PDZ (PSD-95/Disk-large/ZO-1) motif at the extremity of their C-termini (red). The TRPC family can be divided into four subfamilies: TRPC1, TRPC2, TRPC3/6/7 and TRPC4/5. TRPC3, 6 and 7 form a subfamily sharing 70–80% amino acid identity [23,64]. TRPC4 and 5 (sometimes TRPC1 is included in this subfamily) share ~65% homology [65]. The lower scale shows the number of amino acids. The inset to the right shows a schematic representation of TRPC channels anchored in the plasma membrane with three extracellular loops and the predicted pore region between the transmembrane domains 5 and 6.

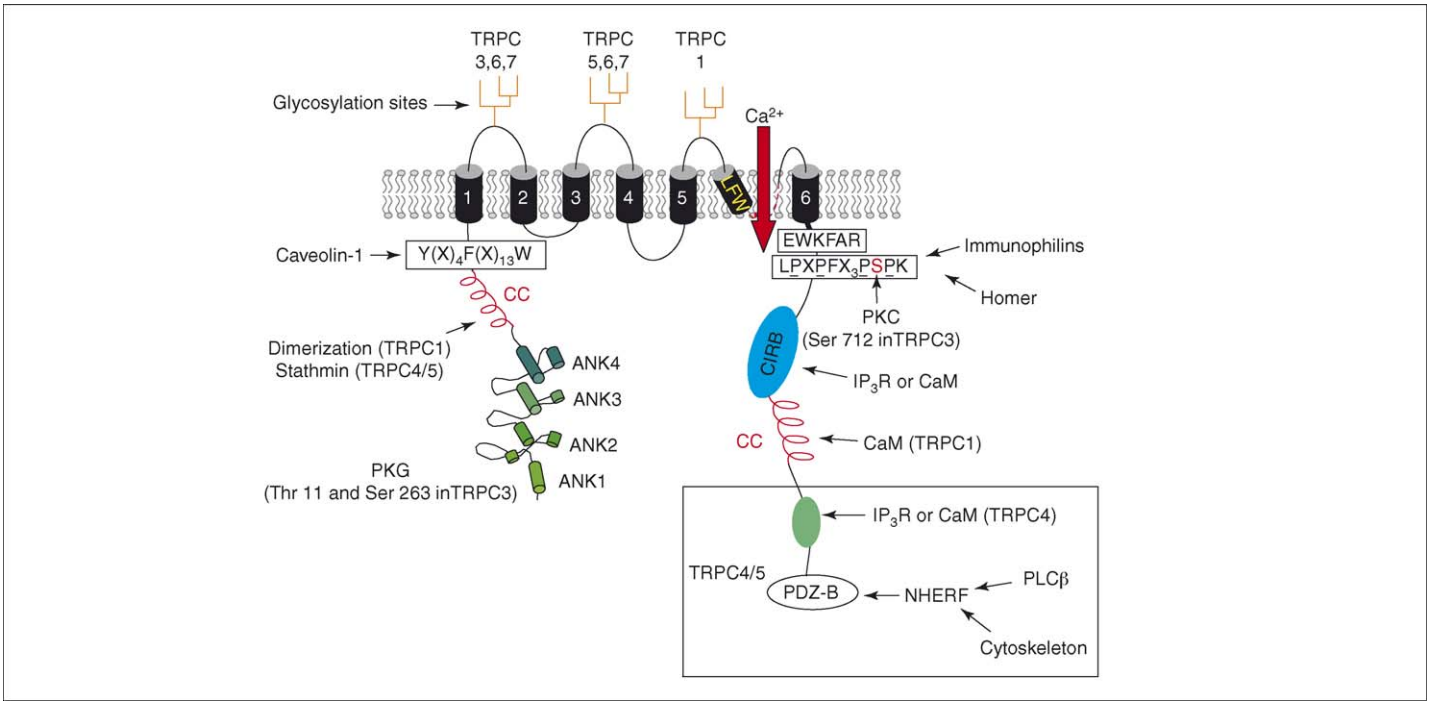

FIGURE 2

Functional protein domains of canonical transient receptor potential channels. The N-terminus of canonical transient receptor potential (TRPC) channels is composed of three to four ankyrin (ANK) repeats, a predicted coiled-coil (CC) region and a putative caveolin-binding region. The cytoplasmic C-terminus includes the transient receptor potential (TRP) signature motif (EWKFAR), a highly conserved proline-rich motif, the calmodulin/IP3 receptor-binding (CIRB) region and a predicted coiled-coil region. An extended C-terminus containing a PSD-95/Disk-large/ZO-1 (PDZ)-binding motif is unique to TRPC4 and TRPC5 (C-terminus area in the box). The ANK repeats appear to be required for correct targeting of TRPC3 to the plasma membrane. The coiled-coil is a ubiquitous protein motif that is commonly used to control oligomerization. CC domains might contribute to oligomerization of TRPC proteins or to mediate TRPC interaction with other proteins. The microtubule destabilizing phosphoprotein stathmin was proposed to bind TRPC5 via the N-terminus CC domain. Colocalization with caveolins has been shown for TRPC1 and TRPC3. A caveolin-binding motif is conserved in all members of the TRPC family located in the cytosolic N-terminus. An LFW motif is located in the predicted pore helix of TRPC5 and TRPC6. A proline-rich motif (LPXPFXXXP**S**PK) downstream of the EWKFAR motif is conserved in all members of the TRPC family and is thought to be responsible for interaction with Homer (for TRPC1) and/or immunophilins FKBP12 and FKBP52. Within this same motif, phosphorylation of Ser 712 in TRPC3 by protein kinase C (PKC), negatively regulates the channels [66]. Similarly, protein kinase G (PKG) inhibits the TRPC3 through phosphorylation on Thr 11 and Ser 263 in the N-terminus [67]. The PDZ-binding motif TRL (Thr-Arg-Leu) of TRPC4 and TRPC5 seems to be responsible for the interaction with the adaptor protein, Na^+/H^+ exchanger regulatory factor (NHERF), possibly linking the channels to phospholipase Cβ (PLCβ) and the cytoskeleton. TRPC6 and TRPC7 appear to be glycosylated in two different loops, whereas the other TRPCs are monoglycosylated. Modified, with permission, from Ref. [21].

molecule in its native environment, remains elusive. The ability of cells to utilize TRPC proteins in diverse combinations highlights the heterogeneity and complexity of Ca^{2+} entry routes in the human organism and the difficulties inherent to the TRPC field. Later in this review, I will discuss the relevance of TRPC channels as potential drug targets in disease therapy focusing mainly on vascular and immune diseases as examples.

Canonical transient receptor potential channels in the vascular system

Alteration of signaling pathways in smooth muscle and endothelial cells largely contributes to hypertension [27]. Hypertension is a risk factor for stroke, myocardial infarction, renal failure, congestive heart failure and progressive atherosclerosis [28].

Ca^{2+} entry into smooth muscle cells is essential for smooth muscle contraction leading to blood vessel constriction. It has been suggested that TRPC6 activation in smooth muscle cells contributes to membrane depolarization and subsequent activation of L-type Ca^{2+} channels [29] that cause vasoconstriction by increased intravascular pressure. TRPC6 was described as a component of the endogenous α1-adrenoceptor-activated nonselective cation channel family in portal vein smooth muscle cells [30], where it plays an important role in regulating vascular tone. Knockdown of TRPC6 led to a decrease of this α1-adrenoceptor-activated nonselective cation current [30]. TRPC6 appears to be a molecular component of vasopressin-stimulated non-selective cation channels in aortic smooth muscle cells [31]. Furthermore, TRPC6 upregulation in response to platelet-derived growth factor (PDGF) was implicated in pulmonary artery smooth muscle proliferation, a determinant of pulmonary hypertension [32]. Surprisingly, TRPC6 knockout ($TRPC6^{-/-}$) mice showed increased vascular smooth muscle contractility. This was attributed to *in vivo* replacement of TRPC6 by constitutively active TRPC3 channels, which resulted in enhanced basal and agonist-induced cation entry into smooth muscle cells, leading to more-depolarized membrane potentials and increased smooth muscle contractility [33]. Yu *et al.* [34] showed that patients with idiopathic pulmonary arterial hypertension (IPAH) caused by excessive smooth muscle cell proliferation exhibit enhanced TRPC3 and TRPC6 expression. Inhibition of TRPC6 expression using RNAi markedly attenuated IPAH and smooth muscle cell proliferation. TRPC6 is highly expressed in the human lung where it might take part in modulation of mucus secretion by bronchial epithelial cells

[25], and might also be involved in bronchial smooth muscle contraction [35,36]. TRPC channels appear to be the major contributors to the contraction of bronchial smooth muscle cells, because these cells do not prominently express L-type Ca^{2+} channels. Therefore, channel blockers against TRPC6 or other TRPC channel isoforms could be useful in bronchodilator therapy for treatment of allergies and asthma. Such drugs might prove useful as an alternative therapy for controlling vascular tone in hypertensive patients.

Recent data showed upregulation of smooth muscle cell TRPC1 channels in animal models of vascular injury and in human vein samples exhibiting intimal structures evoked by coronary artery bypass graft surgery [37]. A TRPC1-specific blocking antibody significantly reduced neointimal growth in human vein, Ca^{2+} entry and proliferation of smooth muscle cells in culture, without apparent effect on endothelial cell function. It was proposed that TRPC1 upregulation is a general feature of smooth muscle cells in occlusive vascular disease, a condition characterized by the switch in smooth muscle cells to an invasive and proliferative mode [37]. It was suggested that TRPC1 inhibitors could potentially be used as protective agents against human vascular failure [37,38].

In endothelial cells, Ca^{2+} entry promotes increase in endothelial permeability and the release of NO, which induces smooth muscle relaxation [39]. TRP channels are widely expressed in the endothelium where they control a variety of physiological functions including vascular permeability, mechanosensing, secretion, angiogenesis, endothelial cell proliferation and apoptosis [40]. Importantly, Ca^{2+} entry in endothelial cells appears to be controlled by different TRPC isoforms. Stimulation of human umbilical vein endothelial cells with tumor necrosis factor–α (TNF-α) led to increased TRPC1 expression accompanied by increased SOC entry in response to thrombin or thapsigargin [41]. Subsequently, these authors showed that the increased expression of TRPC1 in human pulmonary artery endothelial cells in response to TNF-α promotes augmentation of thrombin-induced SOC activation and increased endothelial permeability [42]. Aortic endothelial cells derived from $TRPC4^{-/-}$ mice lack SOC currents, and this strongly correlates with impaired agonist-dependent vasorelaxation [43]. In lung endothelial cells derived from these $TRPC4^{-/-}$ mice, thrombin-receptor-dependent activation of the SOC entry pathway and lung microvascular permeability are significantly reduced [44].

Drugs targeting TRPC4 or TRPC1 could prove to be beneficial in controlling endothelial cell permeability during chronic inflammation. Pocock *et al.* [45] suggested a role for TRPC channels in vascular endothelial growth factor (VEGF)-mediated increase in vascular permeability *in vivo*. It was shown that TRPC1 is involved in VEGF-mediated Ca^{2+} entry in endothelial cells and that angiopoietin-1 opposes VEGF-induced increase in endothelial permeability by inhibiting TRPC1-dependent Ca^{2+} influx [46]. VEGF is a growth factor known to promote endothelial cell permeability and angiogenesis, an important determinant in cancer progression. Drugs targeting TRPC1 can be of use in antiangiogenic cancer therapy and could be an alternative for treatment of pulmonary disease. Indeed, TRPC1 expression is increased in proliferating airway smooth muscle cells and was proposed to play a role in chronic obstructive pulmonary disease and asthma [47–49].

Canonical transient receptor potential channels in the immune system

Disruption of Ca^{2+} homeostasis is known to contribute to neoplastic transformation, apoptosis and anergy of immune cells. T and B lymphocytes are the major players in specific immunity and their successful activation and secretion of cytokines in response to foreign antigens depends largely on Ca^{2+} mobilization. The highly Ca^{2+}-selective SOC current, I_{CRAC}, is the main pathway activated in response to lymphocyte antigen receptor stimulation [50]. This is illustrated by the fact that lymphocytes from patients with primary immunodeficiency lack Ca^{2+} entry and I_{CRAC} in response to antigen stimulation [2].

Although very little data are available on the involvement of TRPC proteins in native SOC entry in leukocytes, it is clear that under certain conditions TRPC1, TRPC3, TRPC5 and TRPC7 can function as SOC channels [7]. Although Orai1/CRACM1 appears to be the pore-forming unit of I_{CRAC} [12], two studies specifically implicated a role of native TRPC1 and TRPC3 as determinants of I_{CRAC} in B and T lymphocytes, respectively. Mori *et al.* [13] showed that genetic disruption of TRPC1 significantly reduces I_{CRAC} and IP_3-mediated Ca^{2+} release from the ER in DT40 B lymphocytes. Consequently, B-cell-antigen receptor-mediated Ca^{2+} oscillations and nuclear factor for activated T cells (NF-AT) activation were reduced in TRPC1-deficient lymphocytes. Philipp *et al.* [14] found that the TRPC3 gene was damaged in Jurkat T cell mutants showing a significant decrease in Ca^{2+} influx and I_{CRAC} in response to T-cell antigen receptor stimulation. The reintroduction of the human TRPC3 into the mutant T cells rescued Ca^{2+} currents as well as the T-cell-receptor-dependent Ca^{2+} entry.

The paramount importance of the SOC pathway in the control of lymphocyte clonal expansion and cytokine secretion makes it an excellent target for novel anti-inflammatory drug therapies. The realization that TRPC channels might be a subunit of SOC channels in lymphocytes offers a molecular target for such drug therapy. More importantly, the molecular heterogeneity of SOC channel makeup and the assumption that different types of leukocytes (e.g. T cells, B cells, neutrophils) might have a different TRPC protein as part of their SOC channels provides an opportunity for selective therapy targeting a single immune cell lineage; this offers the potential to minimize drug side-effects and provides a novel treatment or management for immune-modulated disorders such as autoimmune diseases. Recently, McMeekin *et al.* [51] showed that a SOC entry pathway through a Gq/11-coupled receptor was induced in neutrophils in response to soluble E-selectin and the inflammatory mediator, platelet activating factor (PAF). Because TRPC6 was abundantly expressed by neutrophils, these authors suggested that this SOC pathway is mediated by TRPC6.

In addition, other store-independent Ca^{2+} entry pathways that enhance cell proliferation might be unique to one type of leukocytes. Owing to their specific functions in the immune system, different immune cell lineages are likely to express distinct sets of receptor-operated Ca^{2+} channels. For instance, it has been shown that Ca^{2+} mobilization, as well as lymphocyte proliferation and function, is more dependent on phosphatidylinositol 3-kinase (PI3-K) in B cells than in T cells [52]. The second messenger PIP_3 has been shown to stimulate a non-SOC entry pathway in platelets, RBL-2H3 mast cells and Jurkat T cells [53]. Moreover,

TRPC6 has been identified as a component of the putative PIP_3-sensitive channel in Jurkat T cells [53]. PI3-K signaling promotes proliferation and survival in many cell types including leukocytes, and drugs targeting TRPC6 channels used alone or combined with conventional therapies (i.e. inhibitors of the enzyme topoisomerase IIa) might be useful in strategies aimed at selective control of Ca^{2+}-entry-mediated cell proliferation in relapsed or refractory leukemia patients. Drugs targeting TRPC6 or other TRPC proteins can also be useful in the treatment of autoimmune diseases and during transplant rejection. The relevance of TRPC channels in the treatment of human cancers could extend beyond those of lymphatic origin. A recent report showed that α1-adrenergic receptor stimulation promotes proliferation of primary human prostate cancer epithelial cells by inducing a store-independent Ca^{2+} entry pathway that relied mostly on TRPC6 channels [54]. This further highlights the importance of TRPC channels (and TRPC6 in particular) as novel potential targets in cancer therapy.

As mentioned previously, discovering the exact stoichiometry of each individual TRPC protein within native Ca^{2+} entry channels would yield important information for the design of selective drug therapies for diseases, which involve interactions between different cell types and systems within the organism. An example is inflammation, where a complex network of molecular and cellular interactions takes place, including cytokine secretion by leukocytes and platelets, increased endothelial cell permeability and protein and neutrophil migration.

Drug discovery

The previously described systemic diseases probably involve a complex array of molecular players. Other TRPs might play important roles in the pathology of these diseases. For instance: TRPM8, a Ca^{2+}-permeable channel, seems to have a prominent role in prostate cancer; TRPM8 expression is enhanced in malignant prostate tumors as well as in primary breast, lung, colon and skin cancers; and suppression of TRPM8 expression leads to apoptosis of LNCaP cells (reviewed in [25]). Similarly, TRPV6 expression is increased in prostate cancer, correlates with tumor grade and is a strong indicator of patient outcome [25]. Reciprocally, TRPM1 is downregulated during malignant melanoma and, along with the Ca^{2+}-permeable TRPM2, can act as a tumor suppressor [25]. Although growing data support a prominent role for TRPCs in respiratory diseases, other TRPs might contribute to the exacerbation of these diseases. TRPV1 and TRPV4 are expressed in the airways, where they might be associated with bronchial hyperresponsiveness and could contribute to development of asthma [25]. Clearly, combined drug therapy targeting more than one TRP protein should yield better results than monotherapy. The challenge is to develop compounds that are selective enough to avoid severe side effects.

By analogy with L-type Ca^{2+} channels [55], it is conceivable that potential drugs targeting TRPC channels might act through binding to a common region. Delineating such a region would certainly hasten the discovery and refinement of new compounds for therapeutic use. Although RNAi strategy might be problematic by targeting other proteins it is promising for therapy and should be pursued for future use. In the past, the lack of molecular identity of ROC channels hindered the development of drugs for therapeutic use. ROC channel blockers currently available suffer from the lack of selectivity and lack of high affinity for their targets [35,56]. The emergence of TRPC channels as plausible candidates for ROC channels offers a unique opportunity for drug discovery. How can selective targeting of TRPC proteins for therapeutic use be achieved? The answer to this question lies in combined efforts between scientists in academia and their counterparts in industry. It is important for academic investigators to focus on the following questions: 1) How are TRPC channels activated and regulated under physiological conditions? 2) What is the structure–function relationship of these channels? 3) What is the molecular makeup and stoichiometry of TRPC channels in their native environment?

The quest for specific agents that target TRPC channels for the purpose of therapy is clearly in its infancy. Indeed, basic understanding of TRPC channel regulation and function is relatively limited compared with TRPV and TRPM channels. Likewise, pharmacological agents that are readily available for TRPV and TRPM channels are virtually absent for TRPC family members. Data from the TRPC field are plagued with controversy. There is an inherent difficulty studying membrane proteins in addition to the lack of reliable antibodies against TRPC proteins. For these reasons, serious protein-chemical and structural studies of TRPC channels have not been undertaken and knowledge of the pore structure, as well as the exact gating mechanism of TRPC channels, is lacking. Although it is known that the TRPC3/6/7 subfamily members are activated by DAG and negatively regulated by protein kinase C [23], how DAG activates TRPC3/6/7 is not clear. The mechanism of activation of TRPC1/4/5 through PLC-coupled receptors remains a mystery. TRPC5 activation was shown to depend upon PLC activation, but clearly did not involve DAG or IP_3 [57]. One possibility that remains unexplored is whether PIP_2 exerts a tonic inhibition on TRPC1/4/5 subfamily members and PLC-induced breakdown of PIP_2 relieves this inhibition leading to channel activation. PIP_2 is a key regulator of many ion channels and transporters including TRP channels [58] and one member of the TRPV family, TRPV1, appears to be inhibited by PIP_2 [59].

Studies on *Drosophila* TRP were instrumental is establishing the role of PLC in the activation of these channels [17,60]. Insights from *Drosophila* studies provided evidence for interactions between TRPC channels and other proteins and the regulatory role these interactions play in TRPC channel function and localization into specific cellular microdomains [17,21,60]. Although no one can predict with certainty how long it would take to approach drugs against TRPC channels, further understanding of the mechanism of activation and regulation of TRPC channels, along with discovery of the site of action of second messengers such as DAG and PIP_2 as well as TRPC-interacting partners, would certainly aid drug discovery development. Biochemical and structural studies on purified protein domains should yield useful information towards the exact contribution of each TRPC molecule to native Ca^{2+} channels in mammalian cells. The use of RNAi and knockout animal models combined with functional assays should confirm the physiological contribution of TRPC proteins in native Ca^{2+} entry pathways and validate the relevance of TRPC channels as drug targets for disease therapy.

Current efforts from the drug industry are probably focused on discovering new chemical compounds that affect TRP channels

with high affinity and selectivity. The lessons learned from the voltage-gated Ca^{2+} channel field will doubtless lead to the discovery and refinement of many compounds affecting TRPC channels. The recent availability of systems for high-throughput fluorescence measurement of Ca^{2+} entry and the development of high-throughput patch-clamp techniques will probably speed up the drug screening process. Functional studies will reveal whether compounds targeted against sequences adjacent to the pore region, or the pore region itself, are efficient blockers of TRPC channels. A recent study by Beech and colleagues [61] showed that extracellular application of an anti-TRPC5 antibody targeted against the extracellular loop E3 adjacent to the pore region (see Figure 1) led to specific TRPC5 inhibition with no effect on its closest family members. This example shows that it might well be possible to target TRPC proteins with selectivity and, as a result, offers hope that novel therapies can be developed in the future.

Conclusions

The introduction of new drugs targeting TRPC channels will hopefully improve the management of patients suffering from vascular, pulmonary and immune diseases. It would be difficult to overstate the central importance of Ca^{2+} in biological functions and the importance of modulating Ca^{2+} entry in the treatment of these diseases. Pharmacological blockers of L-type voltage-gated Ca^{2+} channels in smooth muscle cells have been used for decades in the treatment of hypertension and angina [35] and, as such, set a precedent that drugs modulating Ca^{2+} influx can represent useful therapeutic modalities. Clearly, receptor-operated Ca^{2+} channels are functionally important, not only in non-excitable cells but also in electrically excitable cells [1,6,7,18,50]. Unlike the case of voltage-gated Ca^{2+} channels, progress in the receptor-operated Ca^{2+} channel field has been hindered in the past by the lack of molecular candidates for these channels. Although the electrophysiological and pharmacological properties of receptor-operated Ca^{2+} channels are not fully understood, the recent discovery of TRP proteins has provided molecular candidates for these channels and some of the most promising targets for novel drug therapy of human disease. The pathology underlying several diseases has been shown to be accompanied by alterations in Ca^{2+} signaling, including the SOC entry pathway activated through ER Ca^{2+}-store depletion [7]. In addition to cases of primary immunodeficiency mentioned previously, acquired immunodeficiency induced by either viral infection [62] or environmental toxicant exposure, acute pancreatitis [7] and Alzheimer's disease [7] are characterized by altered SOC entry. The number of channelopathies involving a specific defect in the expression or activation of native TRP channels is increasing [25]. Recent studies have linked mutations in the TRPC6 gene to a fatal kidney disease called focal segmental glomerulosclerosis [63]. Three of the six TRPC6 mutations characterized are accompanied by increased channel activation. Future studies will probably incriminate other TRPC channel defects in human disease and further highlight the importance of studying this widely expressed family of cation channels.

The challenge for the future is to employ an integrative approach within an organ or a system to make sense of the ever-growing and seemingly complex information that is available to us. By analyzing the Ca^{2+} entry pathways in different cell types within a system and discovering which set of TRP channels is playing an active role in these pathways we will develop specific drug therapies to reduce side effects and provide better treatment of human diseases.

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