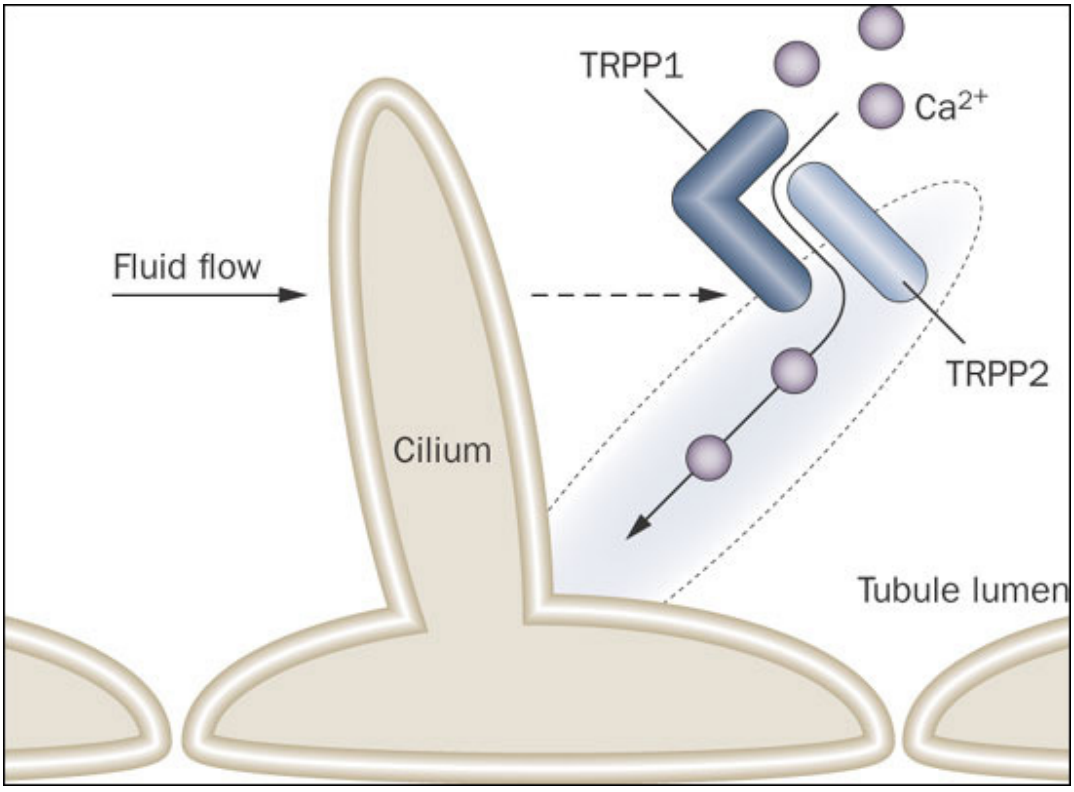
The Current Stage of Drug Development Based upon the Function and Dysfunction of Transient Receptor Potential Channels



Edwin de Jong

Bachelor Biology, Rijksuniversiteit Groningen

Department: Membrane Enzymology

Supervisor: Dr. Armagan Kocer

December 2010

university of groningen

Index

Summary

Precisely controlled movements of ions into and out of cells and organelles are essential for life. TRP channels are a large class of channels that are united by a common primary structure and permeability to monovalent cations and calcium ions. They open and close to regulate cation entry into the cell. TRP channels play a crucial role in all the senses that allow humans to perceive the outside world. Because of the involvement of TRP channels in many physiological processes, it might not be surprising that some of the subfamilies are involved in human diseases. In this review we want to show the current stage in drug development targeting TRP channels in pain, cancer and polycystic kidney disease (PKD). In the management of pain, TRPV1 has become a viable drug target for clinical use. Several synthetic antagonists of the TRPV1 channel are progressed into clinical development focused primarily for use in the treatment of pain. In cancer, TRP channels may serve as prognostic/diagnostic markers or as therapeutic target for activation of the apoptotic pathway or delivering a toxic payload. The disturbance of the interaction between TRPP1 and TRPP2 in PKD has led to the discovery of novel potential therapeutic targets, although the majority of the drugs successfully tested so far were not specifically designed to treat PKD. Last decade, the scientific world opened the gate of TRP channels, but many years of research is required to pass through TRP channels.

Introduction

Precisely controlled movements of ions into and out of cells and organelles are essential for life. In single cells, ion flows mediate crucial processes such as signaling, pH balance, volume regulation, and the cell cycle. In higher organisms, ion flows affect and regulate fertilization, immune responses, secretion, muscle contraction and all of the electrical signals in nerves, muscles and synapses. Ions pass the membrane by going through specific proteins, called channel proteins. Channel proteins can be divided into two classes. The first class is the ion pumps, which transport ions thermodynamically uphill, against the electrochemical potential gradient. [1]

The second class is the ion channels, which transport ions downhill among the electrochemical potential gradient. They are integral membrane proteins that form a pore to allow the passage of specific ions across either plasma membranes or the membranes of intracellular organelles. [2] Ion channels gate in response to specific stimuli, by undergoing conformational changes from closed to open stages, and once open, they allow the passage of thousands of ions. This is in contrast with pumps, which can also transport ions but only one or a few at the time. [3] Many ion channels (such as most sodium, potassium, calcium and some chloride channels) are gated by voltage. Others (such as certain potassium and chloride channels, ryanodine receptors, inositol 1, 4, 5-triphosphate (IP_3) receptors and transient receptor potential (TRP) channels) are relatively voltage-insensitive and are gated by second messengers and other intracellular and/or extracellular mediators. [2]

TRP channels

Transient receptor potential (TRP) channels were first described in *Drosophila*, where photoreceptors carrying TRP gene mutations exhibited a transient voltage response to continuous light. [4, 5] The TRP channels are a large class of channel subunits united by a common primary structure and permeability to monovalent cations and calcium ions, Ca^{2+}. Most TRP channels are relatively nonselective to cations and act to shift the membrane potential to around 0 mV, depolarizing cells from their resting membrane potentials. [6] TRP channels contain six transmembrane segments S1-S6, an intracellular N- and C- terminus, and the pore region loop between transmembrane segments S5 and S6. Four TRP monomers are known to assemble into homotetramers or heterotetramers to form cation channels. [7, 8, 9] (Fig:1) The cytoplasmic ends of the S6 segments form the lower channel, which opens and closes to regulate cation entry into the channel. [10] TRP channels are different from voltage-gated channels because the positively charged residues in S4 of voltage-gated channels are replaced with uncharged amino acid residues in TRP channels. [11, 12] All elements outside the S5-S6 region of a TRP channel provide association or act as linkers to elements that control gating. [10] The highly conserved TRP domain is positioned at the C- terminal site of S6 in most TRPs. [8, 13]

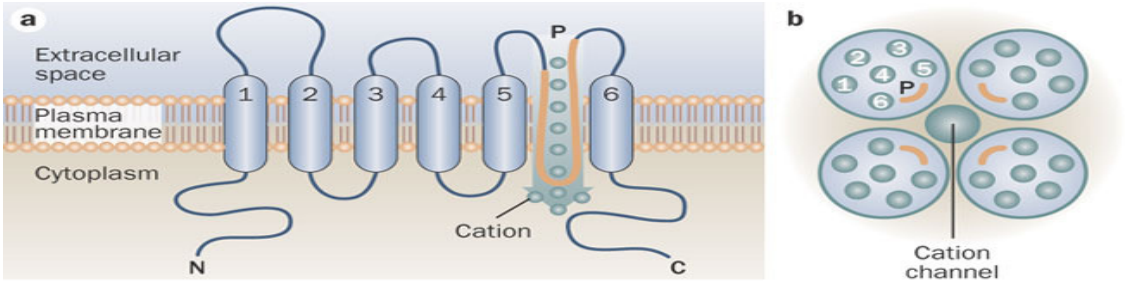


Figure 1: (a) Schematic image of six membrane-spanning segments (S1-S6) of a TRP protein. The pore loop (P) is located between segment S5 and S6, and both the amino (N) and carboxy (C) terminus in the cytoplasmic side of the membrane are depicted. (b) Four TRP monomers assemble into a functional cation channel. [8]

TRP channel proteins are conserved through evolution and are found in most organisms, tissues and cell types. The TRP superfamily is classified into seven related subfamilies based on sequence similarity: "classical" (canonical) TRP channels (TRPC), vanilloid receptor related TRP channels (TRPV), melastatin related channels (TRPM), ankyrin related channels (TRPA), polycystin related channels (TRPP), mucolipin related channels (TRPML), and no mechanopotential related channels (TRPN). [12, 14] Except for TRPN, all the subfamilies can be found in mammals. [12] The mammalian TRP channel family group 1 consist of TRPC, TRPV, TRPM and TRPA, which bear the strongest sequence homology to the founding member of the superfamily, *Drosophila* TRP. TRPC are the classical subfamily because those channels are most related to *Drosophila* TRP. The other subfamilies are so named based on the original name of the initially described member of each subfamily. The two subfamilies TRPP and TRPML comprise the group 2 TRPs, which are distantly related to the group 1 TRPs. The TRPP and TRPML channels share sequence homology over the transmembrane segments but unlike group 1 TRPs, they contain a large loop separating the first two transmembrane domains. [15] (Fig:2) The ankyrin repeats in the N- terminus of TRPC, TRPV and TRPA are mainly involved in mediating protein-protein interactions. [16, 17, 18] The coiled coil domains in TRPC, and in other subfamilies (TRPM), are involved in the activation of the TRP channel and forming of the tetramer. [19, 20]

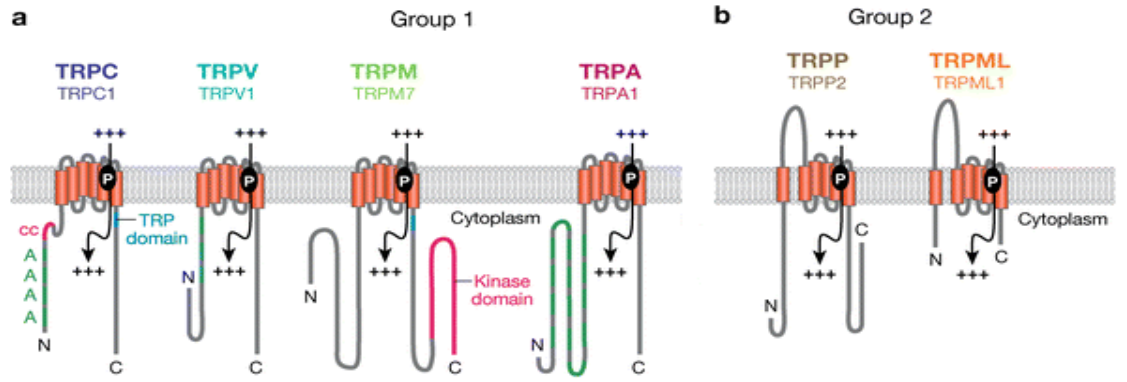


Figure 2: The TRP superfamily in mammals. (a) Single members from each of the four group 1 subfamilies. (b) Single members from each of the two group 2 subfamilies. The following domains are indicated: A, ankyrin repeats; cc, coiled coil domain; protein kinase domain; TRP domain. Also shown are transmembrane segments and pore loop (P), allowing the passage of cations (+++). [15]

TRP channels in the human body

Aristotle defined in De Anima Book II for the first time five exteroceptive senses that allow humans to perceive the outside world: sight, hearing, smell, taste and touch. Balance is now generally considered to be a sixth exteroceptive sense. These senses can be set apart from the so called interoceptive senses, which provide information from within the body. Classifying senses based on the kind of stimulus that is measured, lead to the distinction between photosensation, mechanosensation, chemosensation and thermosensation. Whereas sight (photosensation), hearing and balance (mechanosensation), and smell (chemosensation) easily fit in these four functional categories. Taste can be primarily categorized under chemosensation, but has some clear thermo- and even mechanosensitive aspects too. Touch involves a mixture of thermo- and mechanosensation. All these senses are possible thanks to TRP channels. [21]

TRP channels play a crucial role in photosensation, although they are not involved in the mechanism of light perception in mammalian rods and cones. Apart from the rods and cones, the mammalian eye contains another set of light sensitive cells, the so called intrinsically photosensitive retinal ganglion cells (ipRGCs). These cells are involved in light dependent processes, such as pupil constriction and brightness detection, coupled to circadian rhythms. The ipRGCs express melanopsin, a light sensitive g protein-coupled receptor. [15, 21] When melanopsin and mammalian TRPC3 (member of the TRPC subfamily) were transfected into the HEK293 cell line, these cells attained a light induced current similar to ipRGCs. [22, 23] The exact contribution of particular TRP channels to the light induced activation of ipRGCs has yet to be established. [21]

An example of mechanosensation are the hair cells in the cochlea and vestibular organ which translate mechanical information originating from sound waves or head movement, respectively, into a neural signal that is transported to the central nervous system. [21] The identification of the crucial role for TRPN1 (member of the TRPN subfamily) in mechanosensation in flies and zebrafish encouraged speculation about a potential role of TRP channels in mammalian hearing. [24] TRPA1 (member of the TRPA subfamily), the closest mammalian homologue of TRPN1, was put forward on the basis of its specific localization in the apical region of the inner hair cells and the reduction of transducer after knockdown of TRPN1/TRPA1 expression in zebrafish and mouse hair cells. [21, 25] The molecular architecture of the TRPA1 N-terminus with its multiple ankyrin repeats suggested that it could form the elastic gating spring found in hair cells. [15, 26] (see also Fig:2) However, the auditory and vestibular behaviors and transduction in TRPA1 deficient mice are normal. [27, 28] Other potential TRP channels described in literature are not proved in human, or have more probably indirect effects on mechanosensation. [14, 21]

Chemosensation is mediated by a variety of anatomical structures and sensory systems each being equipped with distinct types of receptor cells. Our perception of food originates from activation of taste receptors on the tongue, olfactory receptor neurons in the nose and trigeminal fibers in the mouth, throat and nose. [21] In taste receptor cells, receptor proteins for bitter, sweet and umami activate phospholipase C, leading to the production of inositol triphosphate and release of Ca^{2+} from intracellular stores. Current

models propose that activation of TRPM5 (member of the TRPM subfamily) by Ca^{2+} released from intracellular stores leads to receptor cell depolarization and subsequent release of ATP. Released ATP then stimulates afferent taste fibers, either directly or via another type of taste cell. [21] TRPM5 null mice showed a reduced/complete loss of response to sweet, bitter and umami stimuli. [29, 30] Recently evidence indicates that this TRPM5 dependent mechanism for the detection of chemical molecules is also used by the olfactory system. [31] TRPP3 (member of the TRPP subfamily) has been implicated in sour taste. Elimination of the cells that express TRPP3 fully abolished sour taste response in mice. [21] One study reported that heterologous co-expression of TRPP3 and a specific other protein resulted in sour activated current responses, suggesting that together these proteins may form a functional sour receptor. [32] Several compounds that we use to spice up our food directly act on the trigeminal neurons. TRP channels which have been identified as the key chemoreceptors in these neurons are: TRPV1 (member of the TRPV subfamily), the receptor for "hot" compounds in different types of pepper; TRPM8 (member of the TRPM subfamily), the receptor for "cool" compounds such as menthol and eucalyptol; and TRPA1, the receptor for pungent compounds, such as mustard oil and cinnamon. [21]

Animals sense temperature, either cold or hot, by the direct activation of temperature sensitive members of the TRP channel families. Mammals can discriminate temperatures ranging from extreme cold (about -10 °C) to extreme heat (about 60 °C). [33] Thermally sensitive TRP channels are each tuned to a distinct temperature range and most are expressed in cutaneous sensory neurons or other cell types in the skin. [34, 35] TRPV1-4 (all members of the TRPV subfamily) are involved in heat transduction while TRPM8 and TRPA1 are involved in cold transduction. For instance, TRPV1 expressing cells are activated by a temperature above 42 °C. The heat gated TRPV2 channel is activated by very high temperatures (above 52 °C). This property suggests a role for TRPV2 in the transduction of painfully hot temperatures, although supporting evidence in vivo has yet to emerge. [33, 36] Two other TRPV subfamily members, TRPV3 and TRPV4, can also be activated by heat. TRPV4 exhibits an apparent threshold of about 27-34 °C, whereas that of TRPV3 is about 32-39 °C. These traits, combined with their expression in skin, make TRPV3 and TRPV4 candidate participants in the perception of warmth. [33] TRPM8 is activated by either modest cooling from normal skin temperature (about 32 °C) to temperatures below about 30 °C. TRPA1 can be activated by the cold at less than 18 °C. Although, recent data show that cold sensation is little affected in TRPA deficient mice, and that a substantial population of sensory neurons responding to cold stimuli does not express either TRPM8 or TRPA1. [27, 28, 37] Another, as yet unidentified cold sensor is therefore more important than TRPA1 in detecting extreme cold in vivo. [36]

Because of the involvement of TRP channels in many physiological processes, it might not be surprising that some of the subfamilies are involved in human diseases. Therefore, affecting the function and treating dysfunction of TRP channels in processes and diseases became new goals in the scientific world since past few years. In this review we want to show the current stage in drug development based upon the function (in pain) and dysfunction (in cancer and polycystic kidney disease) of TRP channels.

TRP channels as target against pain

Pain results from the complex processing of neural signals at different levels of the central nervous system (CNS). Primary sensory neurons are the interface of the nervous system with the external and internal environments that exist outside and inside our bodies. These neurons have a cell body located in the dorsal root ganglion, a peripheral axon that innervates tissues and whose terminals react to sensory stimuli, and a central axon that enters the spinal cord to transfer information to the CNS by synaptic communication. A major function of the sensory apparatus is to detect potentially damaging stimuli and thereby warn the body of the risk of injury. The first neural mediator of this crucial alarm system is the nociceptor, a high threshold primary sensory neuron that detects noxious stimuli. [38]

The threshold that we sense the pain is tightly regulated. It must be high enough so that most activities can be carried out largely pain free, but sensitive enough so that an alert can be given immediately if an injury is impending. For example, a healthy tissue is not painful, however, after an injury the inflammatory response in that tissue sensitizes nociceptors so that their threshold for activation drops and their responsiveness increases, and this contributes to pain hypersensitivity at an inflamed site. [38]

The group of drugs that are used to relieve pain are called as analgesia. They show their effect either by decreasing excitation or increasing inhibition in the nervous system. For instance, opioids decrease neurotransmitter release presynaptically and hyperpolarize neurons postsynaptically in the spinal cord, brainstem and cortex. [39] Sodium channel blocking and potassium channel opening drugs reduce excitation throughout the nervous system, whereas amine uptake inhibitors potentiate the actions of inhibitory transmitters in the spinal cord, brainstem and cortex. [40, 41] Even though the traditional analgesia are good painkillers, the main problem associated with central acting drugs is a higher risk of adverse effects. [38]

An alternative approach for developing novel analgesia is to target the very beginning of the pain pathway and aim treatment at receptors and ion channels that transduce noxious stimuli at the peripheral terminals of nociceptors into electrical activity. The nociceptors are mainly expressed in neurons, thereby potentially reducing the side effects of drugs that specifically act on them. The largest group of noxious stimulus detectors is the TRP channel family. [38] Since the identification of TRPV1 as the molecular target for capsaicin, the active ingredient of chili peppers, these channels have been subject to extensive study by the pharmaceutical industry. [2] TRPV1 has become a viable drug target for clinical use in the management of pain.

The therapeutic value of many TRPV1 agonists, like capsaicin and resiniferatoxin (Fig:3), arises from their ability to reduce electrical activity of TRPV1 containing neurons. Activation of TRPV1 by its agonists leads to membrane depolarization, which in turn results in sodium and calcium channel activation. The associated rise in intracellular Ca^{2+} and associated calcium dependent processes end up in long term inhibition of TRPV1 channel activity. [44] Although pain is produced through the activation of the TRPV1

channel by the agonists before the desensitization, the inactivation of TRPV1 channels reduces sensitivity to noxious stimuli, which can be used with limited efficacy to reduce pain. [38]

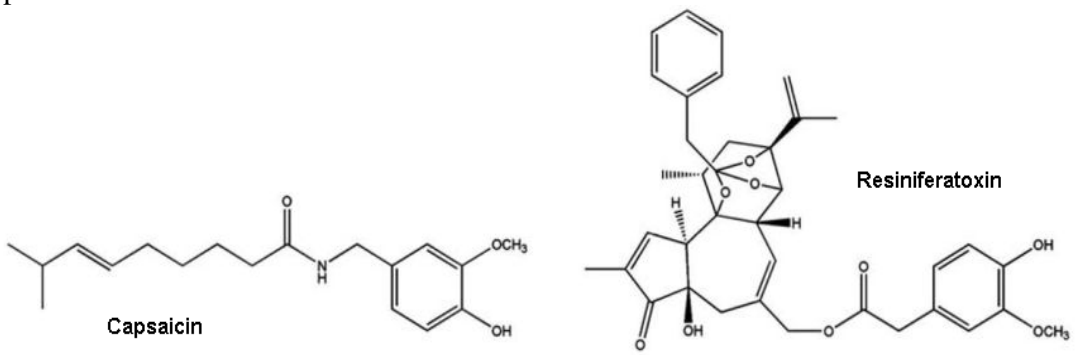


Figure 3: Chemical structures of TRPV1 agonists: capsaicin (the pungent ingredient in chili peppers) and resiniferatoxin (an ultrapotent capsaicin analogue isolated from the cactus-like plant *Euphorbia resinifera Berg*). [42, 43]

Another approach to relieve pain is to block several modes of TRPV1 activation by a group of potential analgesia that are the TRPV1 antagonists. The different elements of an antagonist are highly important for an optimal blocking of the TRPV1 activation. (Fig:4) For example, the hydrogen bonding motif is present in most known TRPV1 antagonist structures. Mono- or bicyclic-aryl and heteroaryl rings with a properly positioned hydrogen bond acceptor in this part of the molecule improve both potency and drug like properties. The lipophilic sidechain interacts with a hypothetical hydrophobic binding site on TRPV1. In addition, proper placement of lipophilic substituents (often 4-CF_3) is crucial for optimal TRPV1 potency. The linkers serve as scaffolding for the proper positioning/spacing of the three elements above, and, therefore, can take many forms, such as direct bonds, single atom or double atom spacers or ring systems. [43]

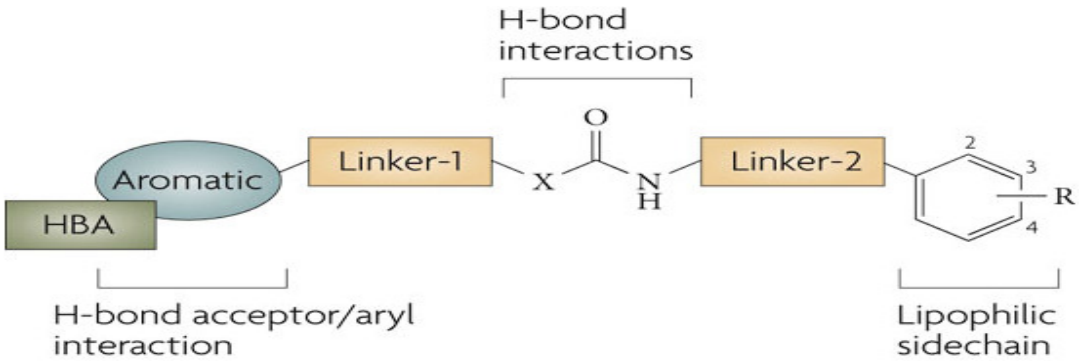


Figure 4: Key binding elements of TRPV1 antagonists. (HBA, hydrogen bond acceptor; X can be nitrogen or carbon) [43]

Several synthetic antagonists of the TRPV1 channel are progressed into clinical development by pharmaceutical companies, like GlaxoSmithKline, Amgen, Abbot, and many others, focused primarily for use in the treatment of pain. (Fig:5) This number of antagonists may be expected to increase in the near future on the basis of the progression of compounds from preclinical development and early stage research. [45] However, potential side effects of TRPV1 antagonists should be considered. For example, recent studies with AMG 517 (Amgen) have demonstrated a long lasting hyperthermia, with core body temperature rising to as much as 40 °C in patients undergoing a dental pain clinical trial. [46]

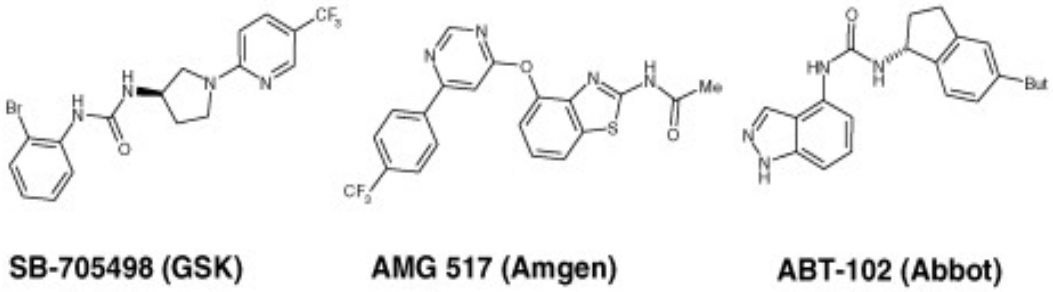


Figure 5: TRPV1 antagonists progressed into clinical development. TRPV1 antagonists are illustrated with associated compound numbers. [45]

TRP channels as target against cancer

Cancer is the second most common cause of death in western countries. [47] It is therefore of fundamental importance to improve the treatment of patients with cancer. The process involved in the transformation of normal cells to tumorigenic cells and tumor progression are complex and only partly understood. This transition is caused by the accumulation of mutations in certain key signaling proteins, along with the formation of those cells capable of competing more aggressively in their local environment and, in the case of metastatic cells, in the environments of other organs. [48] Some Ca^{2+} mediated signaling pathways are also implicated in tumorigenesis and tumor progression, such as metastasis, invasion and angiogenesis. [49]

Ca^{2+} is highly regulated within cellular compartments to achieve the sensitive regulation of cell signaling pathways that can precisely respond to many stimuli. Resting cytosolic free Ca^{2+} is maintained at low levels compared with extracellular free Ca^{2+}. Within the cell itself there is a further Ca^{2+} concentration gradient between the cytosol and some organelles, such as sarcoplasmic/endoplasmic reticulum. These organelles act as Ca^{2+} stores. Unlike the sarcoplasmic/endoplasmic reticulum, the mitochondria is not a large store for Ca^{2+}, however, it may fine tune the Ca^{2+} signal, and its uptake of Ca^{2+} might activate mitochondrial enzymes. Ca^{2+} is often too simplistically viewed as a switch whereby cytosolic free Ca^{2+} is increased by the opening of Ca^{2+} channels on the plasma membrane or on the membranes of internal Ca^{2+} stores, acting as a trigger for Ca^{2+} dependent events. However, a tight control over Ca^{2+} homeostasis is paramount in order to achieve precise control over multiple processes in the same cell, such as regulation of the cell cycle, proliferation or apoptosis. The ability of Ca^{2+} signaling to regulate such

pathways suggests that therapies that modulate Ca^{2+} signaling in cancer cells might be a therapeutic option. Ca^{2+} channels with altered expression in cancer are obvious targets. [49]

TRP channels contribute to changes in intracellular Ca^{2+} concentrations either by acting as Ca^{2+} entry pathways in the plasma membrane or via changes in membrane polarization, modulating the driving force for Ca^{2+} entry mediated by alternative pathways. In addition, TRP channels are expressed on the membranes of internal Ca^{2+} stores, where they may act as triggers for enhanced proliferation, aberrant differentiation, and impaired ability to die, leading to the uncontrolled expansion and invasion characteristic of cancer. Most altered Ca^{2+} expression in cancer involving TRP channels do not involve mutations in the TRP gene, but rather increased or decreased expression levels of the wild type protein, depending on the stage of the cancer. [48] The most clearly described changes are those involving TRPV6 (member of the TRPV subfamily), TRPM1 (member of the TRPM subfamily) and TRPM8. [47, 50] Increased mRNA levels of TRPV6 were found in patient tissue samples from prostate, breast, ovarian, thyroid and colon cancer. [51, 52, 53, 54] TRPM1 is highly expressed in early stage melanomas, but its expression declines with increases in the degree of aggressiveness of the melanoma. [50] Increased mRNA levels of TRPM8 were found in patient tissue samples from melanoma, prostate, breast and lung cancer. [55, 56, 57]

There is no uniform TRP profile for expression changes during carcinogenesis. Hormones, growth factors and alternative splicing isoforms regulate the TRP channel transcription/translation, plasma membrane targeting and localization, as well as the channel activity, resulting in modification of the Ca^{2+} amount entering the cell. [48] (Fig: 6) For instance, TRPM8 expression is regulated by androgens and seems to play a key role in the progression of prostate cancer, which is a hormone dependent cancer. In early stages, prostate cancer progression depends on androgens for growth and survival, and at this time, androgen ablation therapy may cause tumor regression. [58] Anti androgen therapy greatly reduces the expression of TRPM8, confirming that it is regulation by hormones. [48]

The expression of TRPC6 (member of the TRPC subfamily), on the other hand, is regulated by growth factors, which are required to build the vascular and oxygen supplies necessary for tissue growth and survival. [59] The tumor microenvironment produces pro-angiogenic growth factors, such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). VEGF increases intracellular Ca^{2+} concentrations, which modulate microvessel permeability via store independent TRPCs, and in particular TRPC6. Similarly, PDGF mediates up-regulation of TRPC6 expression, and probably, also its function. [48]

Beyond the simple up- and down-regulation of the expression of a particular TRP channel gene by hormones or growth factors, alternative splicing enables the same gene to generate multiple mature mRNA types, resulting in multiple channel proteins. [60] Many of these splice variants are not functional and may not even be efficiently translated. Nevertheless, alternative splicing contributes in the regulation of the expression of functional TRP proteins. This regulation may be achieved by producing nonfunctional

isoforms of the gene by altering the domains necessary for TRP channel opening, membrane localization, or association. Benign melanocytes express the full length TRPM1 mRNA, along with some shorter products. Heterologous co-expression of the full length and short TRPM1 isoforms results in retention of the full length channel in the endoplasmic reticulum. [48]

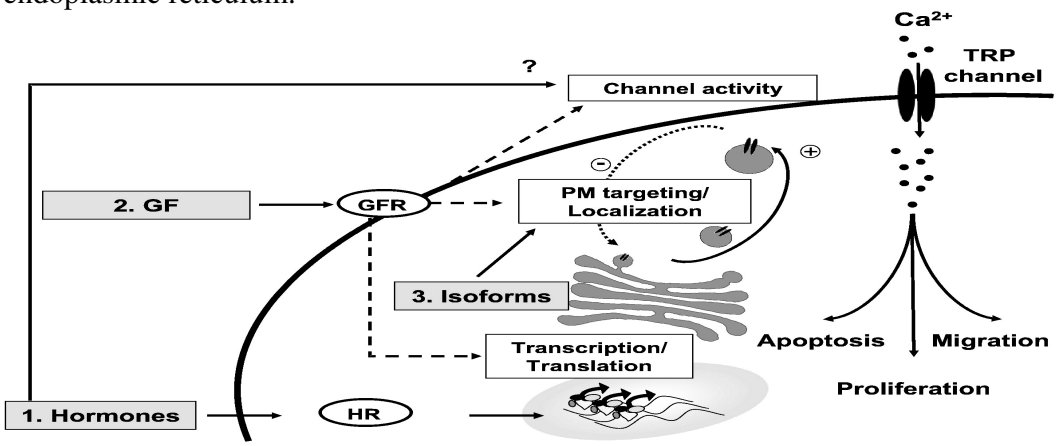


Figure 6: Scheme of the mechanisms through which TRP channels affect tumor growth and metastasis. (growth factor; GF, hormone receptor; HR, GF receptor; GFR, PM; plasma membrane) [48]

Apart from being targets for drug development, some TRP channels may also serve as prognostic or diagnostic markers. TRPM1 has been suggested to be a tumor suppressor and a decrease in TRPM1 expression appears to be a prognostic marker for metastasis in patients with localized malignant melanoma. Similarly, up-regulated TRPM8 and TRPV6 expression in prostate cancer may constitute new diagnostic markers for that disease. [48]

The association of increased or decreased expression of a given TRP channel with cancer and the progression of cancer together with two aspects of the properties of TRP channels 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 sustained high cytosolic Ca^{2+} and Na^{+} concentration, 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. 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, like a radioactive nuclide or toxic chemical, to the cancer cells. Recognition of the TRP channel could be achieved through a tight binding agonist or an anti TRP antibody. [50]

TRP channels as target against polycystic kidney disease

Polycystic kidney disease (PKD), the most common genetic cause of chronic kidney disease, is characterized by the accumulation of numerous fluid filled cysts in the renal parenchyma. The cysts originate from the renal tubules and are lined by a single layer of epithelial cells. Over time, the cysts progressively increase in size due to increased rates of proliferation and active secretion of fluid by the epithelial cells. The enlarging cysts compress surrounding normal nephrons resulting in a decline of renal function. [61] The disease frequently progresses to end-stage renal disease. [8, 62] PKD can be inherited as an autosomal recessive trait or an autosomal dominant trait. The autosomal recessive form of PKD is caused by mutations in the PKHD1 gene, which encodes the protein fibrocystin. [61] The autosomal recessive PKD will not be discussed further in this review because there is no dysfunction of TRP channels involved in this disease. The autosomal dominant form of PKD is caused by mutations in the PKD1 or PKD2 genes, which encodes for the proteins TRPP1 and TRPP2 (members of the TRPP subfamily), respectively. [61, 63] Although 85% of cases are due to PKD1 mutations, disease associated with PKD1 or PKD2 mutations is clinically identical, suggesting that these two genes act in the same molecular pathway. [64]

Studies have focused on localization of the TRPP1/TRPP2 complex to primary cilia and its role in mediating Ca^{2+} fluxes in response to mechanical stimuli. In contrast to the nonselective cation channel TRPP2, TRPP1 is a large integral membrane protein that structurally resembles a receptor or adhesion molecule. TRPP1 and TRPP2 form a protein complex in cilia, which are microtubule based organelles that function as cellular mechanosensors. Flow of fluid induces bending of the cilium on an epithelial cell. This bending causes a formation of a TRPP1/TRPP2 plasma membrane channel, and therefore an increase in intracellular Ca^{2+} levels, which regulates cell signaling pathways. [8, 61, 65] (Fig: 7) In vitro experiments with primary cilia from renal epithelial cells confirmed that TRPP1 and TRPP2 interact physically and functionally to act as a mechanosensor. [66] TRPP2 homomeric channels are not mechanosensitive. [67] These data suggest that malfunctioning of primary cilia, more specifically a disturbance in the interaction between TRPP1 and TRPP2, is involved in PKD. [8]

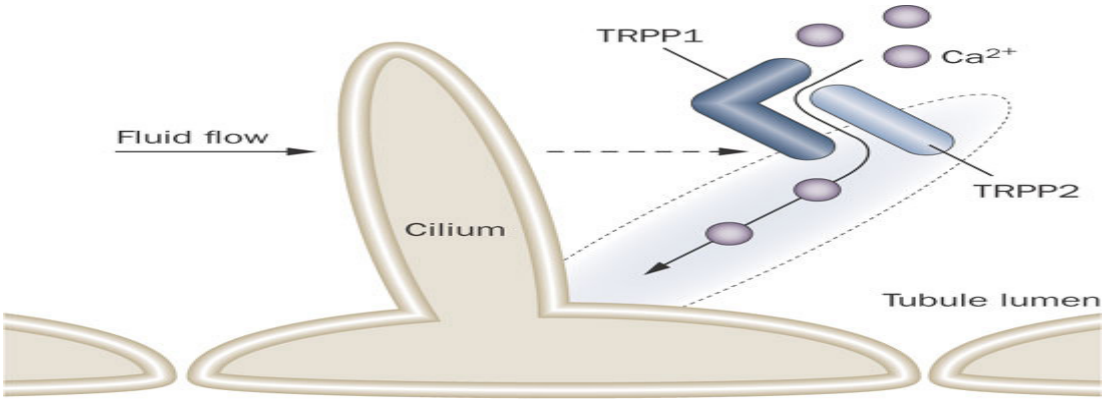


Figure 7: Fluid flow induces bending of the cilium on an epithelial cell, as indicated by the dashed line. This bending causes TRPP1 and TRPP2 to form a complex at the plasma membrane, which allows influx of Ca^{2+} ions. [8]

The influx of Ca^{2+} across the plasma membrane constitutes the initial response to mechanical stimuli, and downstream signaling is mediated through intracellular Ca^{2+} release which regulates numerous molecular activities inside the cell that contribute to tissue development. [66] In PKD, when TRPP1 or TRPP2 are inactivated, the formation and growth of cysts is accompanied by increased proliferation and apoptosis of cyst lining epithelia, loss of epithelia polarity and de-differentiation, dysregulation of cell/matrix interactions and transformation of the absorptive epithelial phenotype to a secretory phenotype. [68]

The contribution of inactivated TRP channels followed by disturbed downstream signaling to epidermal growth factor (EGF), transforming growth factor (TGF) alpha and EGF receptor (EGFR) levels in the cell remains unclear. Nevertheless, EGF, TGF and EGFR play important roles in promoting cystic epithelial proliferation. [68, 69] Cystic fluid is derived from glomerular filtrate in the early stages of autosomal dominant PKD, when cysts are still attached to the parent tubule. Cysts separated from the tubule of origin continue to expand through a transepithelial chloride secretion mechanism mediated by cAMP. In vitro models of cystogenesis demonstrated that expression of TRPP1 at cell-cell junctions at controlled levels is critical for proper tubular differentiation. [70] Alterations in TRPP1 mediated adhesion may cause the abnormal epithelial cell phenotype observed in autosomal dominant PKD cells, including de-differentiation and loss of epithelial polarity. [68] Apoptosis is also essential for cystogenesis, altered expression of pro-apoptotic and anti-apoptotic in mice results in renal cyst formation. [68, 71]

Greater understanding of the molecular mechanism of cyst formation and growth has led to the discovery of novel potential therapeutic targets. However, the majority, if not all, of the drugs successfully tested in animal models so far were not specifically designed to treat PKD but rather were originally developed for other indications. [68]

Conclusion

Now we have seen the current stage of drug development based upon TRP channels in a few diseases, we can conclude that there is a huge difference in development from disease to disease. In the treatment of pain there are several potential analgesia, which recently reached or in near future might reach the clinical phase. As far as we know from the literature, there is no drug development based upon TRP channels for PKD at all. An explanation for the reticence of drug development in PKD compared to pain might be the difference in the basics of the problem. TRP channels in pain are functioning proper. Potential analgesia increase activation to get desensitization or decrease/block the activation of TRP channels to reduce pain. It is not optional to alter the activation of TRP channels related to PKD because there is no functional TRP channel due to a disturbance in the interaction between different TRP channels.

Drug development based upon TRP channels in cancer is in between drug development of pain and PKD. There are some optional targets for drugs against cancer, like Ca^{2+} and Na^{+} entry through TRP channels which leads to sustained high cytosolic Ca^{2+} and Na^{+} concentration, which kills the cells by apoptosis and necrosis. But this strategy requires the selective expression and activation of a given TRP channel in the targeted cancer cells, because Ca^{2+} signaling is involved in many processes in the human body. Other opportunities are to make use of the high expression of some TRP channels in cancer cells to provide a target for delivering a toxic payload to the cancer cell and use TRP channels as prognostic/diagnostic markers. A lot of wonderful optional targets based upon TRP channels, but no drug yet.

It is magnificent to see how much research has been done on TRP channels during the last decade. Nevertheless, the development of drugs targeting TRP channels is still in its infancy at this moment. If we want to get full potential out of TRP channels in the future, the scientific world must focus on the fundamental research involved TRP channels. In my opinion, there must be many more opportunities to use TRP channels in the treatment of diseases. But at this moment, we do not know enough about the TRP channels involved mechanisms of action and the signaling pathways to work efficiently on drug development based upon the function and dysfunction of TRP channels. Last decade, the scientific world opened the gate of TRP channels, but many years of research is required to pass through TRP channels.

Acknowledgements

I would like to give special thanks to Dr. Armagan Kocer for her supervision, assistance and advice during my work on this review about the current stage of drug development based upon the function and dysfunction of TRP channels.

References

Picture on titlepage: Woudenberg-Vrenken TE, Bindels RJ, Hoenderop JG: The role of transient receptor potential channels in kidney disease. Nat Rev Nephrol 5(8): 441-449, 2009

1. Gadsby DC: Ion channels versus ion pumps: the principal difference, in principle. Nat Rev Cell Biol 10(5): 344-352, 2009

2. Mathie E: Ion channels as novel therapeutic targets in the treatment of pain. J Pharm Pharmacol 62(9): 1089-1095, 2010

3. Jentsch TJ, Hübner CA, Fuhrmann JC: Ion channels: function unravelled by dysfunction. Nat Cell Biol 6(11): 1039-1047, 2004

4. Minke B: Drosophila mutant with a transducer defect. Biophys Struct Mech 3(1): 59-64, 1977

5. Montell C, Jones K, Hafen E, Rubin G: Rescue of the Drosophila phototransduction mutation trp by germline transformation. Science 230(4729): 1040-1043, 1985

6. Clapham DE, Runnels LW, Strübing C: The TRP ion channel family. Nat Rev Neurosci 2(6): 387-396, 2001

7. Dimke H, Hoenderop JG, Bindels RJ: Molecular basis of epithelial Ca2+ and Mg2+ transport: Insights from the TRP channel family. J Physol (Epub ahead of print), 2010

8. Woudenberg-Vrenken TE, Bindels RJ, Hoenderop JG: The role of transient receptor potential channels in kidney disease. Nat Rev Nephrol 5(8): 441-449, 2009

9. Schaefer M: Homo- and heteromeric assembly of TRP channel subunits. Pflugers Arch 451(1): 35-42, 2005

10. Clapham DE: TRP channels as cellular sensors. Nature 426(6966): 517-524, 2003

11. Reaves BJ, Wolstenholme AJ: The TRP superfamily: insights into how structure, protein-lipid interactions and localization influence function. Biochem Soc Trans 35(Pt 1): 77-80, 2007

12. Minke B: The history of the drosophila TRP channel: The birth of a new channel superfamily. J Neurogenet 24(4): 216-233, 2010

13. Padinjat R, Andrews S: TRP channels at a glance. J Cell Sci 117(Pt 24): 5707-5709, 2004

14. Christensen AP, Corey DP: TRP channels in mechanosensation: direct or indirect activation. Nat Rev Neurosci 8(7): 510-521, 2007

15. Venkatachalam K, Montell C: TRP channels. Annu Rev Biochem 76: 387-417, 2007

16. Schindl R, Frischauf I, Kahr H, Fritsch R, Krenn M, Derndl A, Vales E, Muik M, Derler I, Groschner K, Romanin C: The first ankyrin-like repeat is the minimum indispensable key structure for functional assembly of homo- and heteromeric TRPC4/TRPC5 channels. Cell Calcium 43(3): 260-269, 2008

17. Lishko PV, Procko E, Jin X, Phelps CB, Gaudet R: The ankyrin repeats of TRPV1 bind multiple ligands and modulate channel sensitivity. Neuron 54(6): 905-918, 2007

18. Nilius B, Prenen J, Owsianik G: Irritating channels: the case of TRPA1. J Physiol (Epub ahead of print), 2010

19. Lepage PK, Lussier MP, Barajas-Martinez H, Bousquet SM, Blanchard AP, Francoeur N, Dumaine R, Boulay G: Identification of two domains involved in the assembly of transient receptor potential canonical channels. J Biol Chem 281(41): 30356-30364, 2006

20. Phelps CB, Gaudet R: The role of the N terminus and transmembrane domain of TRPM8 in channel localization and tetramerization. J Biol Chem 282(50): 36474-36480, 2007

21. Damann N, Voets T, Nilius B: TRPs in our senses. Curr Biol 18(18): R880-889, 2008

22. Qiu X, Kumbalasiri T, Carlson SM, Wong KY, Krishna V, Provencio I, Bernson DM: Induction of photosensitivity by heterologous expression of melanopsin. Nature 433(7027): 745-749, 2005

23. Panda S, Nayak SK, Campo B, Walker JR, Hogenesch JB, Jegla T: Illumination of the melanopsin signaling pathway. Science 307(5709): 600-604, 2005

24. Walker RG, Willingham AT, Zuker CS: A Drosophila mechanosensory transduction channel. Science 287(5461): 2229-2234, 2000

25. Corey DP, Garcia-Anoveros J, Holt JR, Kwan KY, Lin SY, Vollrath MA, Amalfitano A, Cheung EL, Derfier BH, Duggan A, Géléoc GS, Gray PA, Hoffman MP, Rehm HL, Tamasauskas D, Zhang DS: TRPA1 is a candidate for

the mechanosensitive transduction channel of vertebrate hair cells. Nature 432(7018): 723-730, 2004

26. Kung C: A possible unifying principle for mechanosensation. Nature 436(7051): 647-654, 2005

27. Bautista DM, Jordt SE, Nikai T, Tsuruda PR, Read AJ, Poblete J, Yamoah EN, Basbaum AL, Julius D: TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell 124(6): 1269-1282, 2006

28. Kwan KY, Allchorne AJ, Vollrath MA, Christensen AP, Zhang DS, Woolf CJ, Corey DP: TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. Neuron 50(2): 277-289, 2006

29. Damak S, Rong M, Yasumatsu K, Kokrashvili Z, Perez CA, Shigemaru N, Yoshida R, Mosinger B Jr, Glendinning JI, Ninomiya Y, Margolskee RF: Trpm5 null mice respond to bitter, sweet and umami compounds. Chem Senses 31(3): 253-264, 2006

30. Zhang Y, Hoon MA, Chandrashekar J, Mueller KL, Cook B, Wu D, Zuker CS, Ryba NJ: Coding for sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. Cell 112(3): 293-301, 2003

31. Lin W, Margolskee R, Donnert G, Hell SW, Restrepo D: Olfactory neurons expressing transient receptor potential channel M5 (TRPM5) are involved in sensing semiochemicals. Proc Natl Acad Sci USA 104(7): 2471-2476, 2007

32. Ishimaru Y, Inada H, Kubota M, Zhuang H, Tominaga M, Matsunami H: Transient receptor potential family members PKD1L3 and PKD2L1 form a candidate sour taste receptor. Proc Natl Acad Sci USA 103(33): 12569-12574, 2006

33. Lumpkin EA, Caterina MJ: Mechanisms of sensory transduction in the skin. Nature 445(7130): 858-865, 2007

34. Dhaka A, Viswanath V, Patapoutian A: Trp ion channels and temperature sensation. Annu Rev Neurosci 29: 135-161, 2006

35. Lee H, Caterina MJ: TRPV channels as thermosensory receptors in epithelial cells. Pflugers Arch 451(1): 160-167, 2005

36. Huang J, Zhang X, McNaughton PA: Modulation of temperature-sensitive TRP channels. Semin Cell Dev Biol 17(6): 638-645, 2006

37. Babes A, Zorzon D, Reid G: A novel type of cold-sensitive neuron in rat dorsal root ganglia with rapid adaptation to cooling stimuli. Eur J Neurosci 24(3): 691-698, 2006

38. Patapoutian A, Tate S, Woolf CJ: Transient receptor potential channels: targeting pain at the source. Nat Rev Drug Discov 8(1): 55-68, 2009

39. Finnegan TF, Chen SR, Pan HL: Effect of the {mu} opioid on excitatory and inhibitory synaptic inputs to periaqueductal gray-projecting neurons in the amygdala. J Pharmacol Exp Ther 312(2): 441-448, 2005

40. Mico JA, Ardid D, Berrocoso E, Eschalier A: Antidepressants and pain. Trends Pharmacol Sci 27(7): 348-354, 2006

41. Wood JN: Ion channels in analgesia research. Hanb Exp Pharmacol 177: 329-358, 2007

42. Phillips E, Reeve A, Bevan S, McIntyre P: Identification of species-specific determinants of the action of the antagonist capsazepine and the agonist PPAHV on TRPV1. J Biol Chem 279(17): 17165-17172, 2004

43. Szallasi A, Cortright DN, Blum CA, Eid SR: The vanilloid receptor TRPV1: 10 years from channel cloning to antagonist proof-of-concept. Nat Rev Drug Discov 6(5): 357-372, 2007

44. Jara-Oseguera A, Simon SA, Rosenbaum T: TRPV1: on the road to pain relief. Curr Mol Pharmacol 1(3): 255-269, 2008

45. Gunthorpe MJ, Chizh BA: Clinical development of TRPV1 antagonists: targeting a pivotal point in the pain pathway. Drug Discov Today 14(1-2): 56-67, 2009

46. Gavva NR, Treanor JJ, Garami A, Fang L, Surapaneni S, Akrami A, Alvarez F, Bak A, Darling M, Gore A, Jang GR, Kesslak JP, Ni L, Norman MH, Palluconi G, Rose MJ, Salfi M, Tan E, Romanovsky AA, Banfield C, Davar G: Pharmalogical blockade of the vanilloid receptor TRPV1 elicits marked hyperthermia in humans. Pain 136(1-2): 202-210, 2008

47. Bödding M: TRP proteins and cancer. Cell Signal 19(3): 617-624, 2007

48. Gkika D, Prevarskaya N: Molecular mechanisms of TRP regulation in tumor growth and metastasis. Biochim Biophys Acta 1793(6): 953-958, 2009

49. Monteith GR, McAndrew D, Faddy HM, Roberts-Thomson SJ: Calcium and cancer: targeting Ca2+ transport. Nat Rev Cancer 7(7): 519-530, 2007

50. Prevarskaya N, Zhang L, Barritt G: TRP channels in cancer. Biochim Biophys Acta 1772(8): 937-946, 2007

51. Zhuang L, Peng JB, Tou L, Takanaga H, Adam RM, Hediger MA, Freeman MR: Calcium-selective ion channel, CaT1, is apically localized in gastrointestinal tract epithelia and is aberrantly expressed in human malignancies. Lab Invest 82(12): 1755-1764, 2002

52. Fixemer T, Wissenbach U, Flockerzi V, Bonkhoff H: Expression of the Ca2+-selective cation channel TRPV6 in human prostate cancer: a novel prognostic marker for tumor progression. Oncogene 22(49): 7858-7861, 2003

53. Wissenbach U, Niemeyer BA, Fixemer T, Schneidewind A, Trost C, Cavalie A, Reus K, Meese E, Bonkhoff H, Flockerzi V: Expression of CaT-like, a novel calcium-selective channel, correlates with the malignancy of prostate cancer. J Biol Chem 276(22): 19461-19468, 2001

54. Peng JB, Zhuang L, Berger UV, Adam RM, Williams BJ, Brown EM, Hediger MA, Freeman MR: CaT1 expression correlates with tumor grade in prostate cancer. Biochem Biophys Res Commun 282(3): 729-734, 2001

55. Tsavaler L, Shapero MH, Morkowski S, 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(9): 3760-3769, 2001

56. Fuessel S, Sickert D, Meye A, Klenk U, Schmidt U, Schmitz M, Rost AK, Weigle B, Kiessling A, Wirth MP: Multiple tumor marker analysis (PSA, hK2, PSCA, trp-p8) in primary prostate cancers using quantitative RT-PCR. Int J Oncol 23(1): 221-228, 2003

57. Schmidt U, Fuessel S, Koch R, Baretton GB, Lohse A, Tomasetti S, Unversucht S, Froehner M, Wirth MP, Meye A: Quantitative multi-gene expression profiling of primary prostate cancer. Prostate 66(14): 1521-1534, 2006

58. Feldman BJ, Feldman D: The development of androgen-independent prostate cancer. Nat Rev Cancer 1(1): 34-45, 2001

59. Reynolds AR, Kyprianou N: Growth factor signalling in prostatic growth: significance in tumour development and therapeutic targeting. Br J Pharmacol 147(2): S144-S152, 2006

60. Stamm S, Ben-Ari S, Rafalska I, Tang Y, Zhang Z, Toiber D, Thanaraj TA, Soreq H: Function of alternative splicing. Gene 344: 1-20, 2005

61. Patel V, Chowdhurry R, Igarashi P: Advances in the pathogenesis and treatment of polycystic kidney disease. Curr Opin Nephrol Hypertens 18(2): 99-106, 2009

62. Delmas P: Polycystins: from mechanosensation to gene regulation. Cell 118(2): 145-148, 2004

63. Hsu YJ, Hoenderop JG, Bindels RJ: TRP channels in kidney disease. Biochim Biophys Acta 1772(8): 928-936, 2007

64. Qamar S, Vadivelu M, Sandford R: TRP channels and kidney disease: lessons from polycystic kidney disease. Biochem Soc Trans 35(Pt 1): 124-128, 2007

65. Praetorius HA, Spring KR: Bending the MDCK cell primary cilium increases intracellular calcium. J Membr Biol 184(1): 71-79, 2001

66. Nauli SM, Alenghat FJ, Luo Y, Williams E, Vassilev P, Li X, Elia AE, Lu W, Brown EM, Quinn SJ, Ingber DE, Zhou J: Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. Nat Genet 33(2): 129-137, 2003

67. Giamarchi A, Padilla F, Coste B, Raoux M, Crest M, Honoré E, Delmas P: The versatile nature of the calcium-permeable cation channel TRPP2. EMBO Rep 7(8): 787-793, 2006

68. Ibraghimov-Beskrovnaya O, Bukanov N: Polycystic kidney disease: from molecular discoveries to targeted therapeutic strategies. Cell Mol Life Sci 65(4): 605-619, 2008

69. Belibi FA, Edelstein CL: Novel targets for the treatment of autosomal dominant polycystic kidney disease. Expert Opin Investig Drugs 19(3): 315-328, 2010

70. Bukanov NO, Husson H, Dackowski WR, Lawrence BD, Clow PA, Roberts BL, Klinger KW, Ibraghimov-Beskrovnaya O: Functional polycystin-1 expression is developmentally regulated during epithelial morphogenesis in vitro: downregulation and loss of membrane localization during cystogenesis. Hum Mol Genet 11(8): 923-936, 2002

71. Lin HH, Yang TP, Jiang ST, Yang HY, Tang MJ: Bcl-2 overexpression prevents apoptosis-induced Madin-Carby canine kidney simple epithelial cyst formation. Kidney Int 55(1): 168-178, 1999