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TRPA1 as a therapeutic target for nociceptive pain

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ABSTRACT

Introduction: Chronic pain affects approximately 30–50% of the population globally. Pathologies such as migraine, diabetic neuropathy, nerve injury and treatment with chemotherapeutic agents, can induce chronic pain. Members of the transient receptor potential (TRP) channels, including the TRP ankyrin 1 (TRPA1), have a major role in pain.

Areas covered: We focus on TRPA1 as a therapeutic target for pain relief. The structure, localization, and activation of the channel and its implication in different pathways to signal pain are described. This paper underlines the role of pharmacological interventions on TRPA1 to reduce pain in numerous pain conditions. We conducted a literature search in PubMed up to and including July 2020.

Expert opinion: Our understanding of the molecular mechanisms underlying the sensitization of central and peripheral nociceptive pathways is limited. Preclinical evidence indicates that, in murine models of pain diseases, numerous mechanisms converge on the pathway that encompasses oxidative stress and Schwann cell TRPA1 to sustain chronic pain. Programs to identify and develop treatments to attenuate TRPA1-mediated chronic pain have emerged from this knowledge. Antagonists explored as a novel class of analgesics have a new and promising target in the TRPA1 expressed by peripheral glial cells.

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(TRPA1)

1. Introduction – Transient Receptor Potential channels

Transient Receptor Potential (TRP) channels belong to a large family of ion channels with more than 50 subtypes, mostly conserved from nematodes to humans, where they play an important role in homeostatic functions [1,2]. TRPs are grouped in 7 subfamilies based on their amino acid sequence homology: canonical or classic (TRPC1-7), vanilloid (TRPV1-6), melastatin (TRPM1-8), non-mechanoreceptor potential C (NOMP-like, TRPN1), long TRP ankyrin (a solitary member is the transmembrane protein 1 [TRPA1]), and the more distant relatives, polycystins (TRPP1-5) and mucolipins (TRPML1-3) [2,3]. An eighth sub-family labeled TRP yeast (TRPY) is not included in either of these groups because of its distant relation with the other channels [4] (Figure 1). In mammals, the TRP superfamily consists of 6 subfamilies and 28 members that mainly act as nonselective cation permeable channels.

TRPs possess a primary structure that is common to all members, consisting of six transmembrane domains (TM1-TM6), and one hydrophilic loop between TM5 and TM6, forming the pore permeable to monovalent cations and calcium ions [2]. Both the amino (NH₂) and carboxy (COOH) -terminal regions, of variable length, face the intracellular side [5]. Whereas the COOH-terminus possesses highly conserved domains, in most TRP channels, the NH₂-terminus region is characterized by the presence of ankyrin repeats (33-residue motifs consisting of pairs of antiparallel α -helices connected by β -hairpin motifs), which are associated to different features,

such as the regulation of channel assembly in tetramers and interaction with ligands and protein partners [6]. It has been reported that the functional TRP channels are formed by four subunits of a complete TRP structure assembled in a homo- and hetero- tetramer, and heteromultimers [7]. As TRP channels are permeable to calcium and other monovalent ions, they allow an inward cation current that can modulate cell function, mainly intracellular calcium-dependent pathways [6]. In addition, TRP channels can act as integrators of several signaling systems, like those modulated by cell surface receptors, including G protein-coupled receptors (GPCRs) and growth factor receptors [8]. In summary, beyond the structure and cation permeability, TRPs possess an impressive variety of activation modes by mechanical, thermal and chemical stimuli, such as exogenous chemical compounds, lipids, oxidative stress, acids, pheromones, osmolarity, mechanical stimulation, light, and temperature [9]. In addition, regulatory mechanisms, such as transcription, alternative splicing, glycosylation and phosphorylation, and tissue distribution, modulate their activity.

Here, we summarize recent findings regarding the implication of the TRPA1 channel in sustaining pain transmission. To provide a complete overview on the role of TRPA1 as a therapeutic target for chronic pain, a robust literature search was performed on PubMed. To identify the main findings in the field, we used the following search terms: TRPA1, TRPA1 activation, TRPA1 and nociceptive pain, neuropathic pain, cancer pain and migraine.

Article highlights

- TRP channels belong to a large family of ion channels with pleiotropic roles in a variety of cells and systems.
- The TRPA1 channel is widely studied in the mechanism/process of inflammatory, neuropathic, cancer, and migraine pain.
- In addition to the peripheral nervous system, the TRPA1 channel is localized in several non-neuronal cell types.
- Endogenous molecules produced by various pathological conditions, such as reactive oxygen (ROS), nitrate (RNS), and carbonylic (RCS) species, activate TRPA1 to sustain chronic pain.
- TRPA1 activation leads to pain responses.

This box summarizes key points contained in the article.

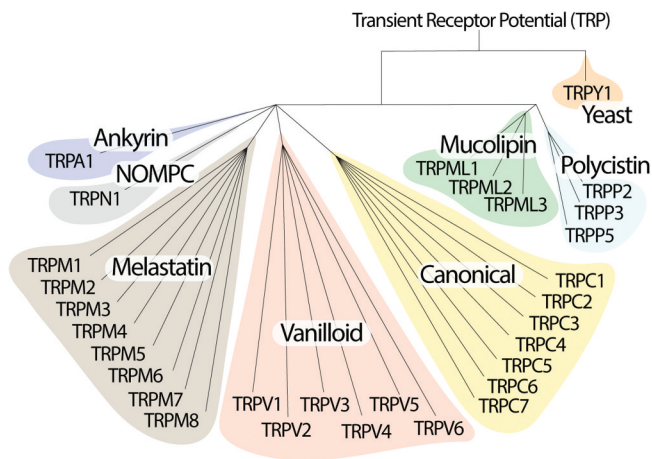


Figure 1. Phylogenetic tree of the eukaryote TRP ion channels superfamily.

2. Transient Receptor Potential Ankyrin 1 (TRPA1)

The history of the TRPA1 channel started in 1999 when it was first identified in lung fibroblasts [10], and was named 'ankyrin-like protein with transmembrane domains protein 1' (ANKTM1). Only later was it recognized as a TRP member for its homology with other components of the channel superfamily [11]. The human *trpa1* gene is located in chromosome 8q13, consists of 27 exons, and spans 55,701 base pairs [10]. One particularity of this receptor is that the *trpa1* gene is found in many animal species (vertebrates and invertebrates), including *Caenorhabditis elegans*, fruit flies, zebrafish, chickens, mice, rats and dogs, in addition to humans. One curiosity is that, while in mammals only one gene for this receptor is present, other animal classes contain multiple TRPA1 homologues (4 in fruit fly, 2 in *C. elegans*, 2 in zebrafish, 4 in the sea squirt, *Ciona intestinalis*) [1,12].

Like other TRPs, TRPA1 possesses the NH₂ and COOH-terminal parts facing the intracellular space. Its name derives from an elongated number (14–19) of an ankyrin repeat region within the NH₂-terminal. The receptor is a nonselective channel permeable to calcium, sodium, and potassium, with a much higher permeability to calcium compared to other TRPs. TRPA1 functions as a sensor of cell damage signals and takes part in many diseases. TRPA1 is implicated in cold sensations related to painful stimuli [13].

Several studies modulating TRPA1 activity have shown that this receptor is involved in inflammatory and immune responses, and in the conversion of physical and chemical stimuli in irritative (itching) or pain sensations [14–16].

2.1. TRPA1 structure

The structure of human TRPA1 has been determined using cryoelectron microscopy [17]. The mostly conserved COOH-terminal region contains positively charged domains for interaction with negatively charged ligands, including inorganic polyphosphates or phosphoinositides, able to modulate TRPA1 activation [18]. As mentioned, on the NH₂-terminal portion, which constitutes more than half of the protein (around 64%) [19], TRPA1 possesses an elongated ankyrin repeat domain (ARD 14–18) with the role of controlling the protein-protein interaction, as well as the channel insertion and regulation in the plasma membrane [20,21]. The initial region of the NH₂-terminal portion of the protein, located before the first transmembrane segment, is responsible for allosteric regulation of TRPA1 and consists of a prominent ankyrin repeat domain (amino acids 1–639) and a linker (amino acids 640–720), formed by a β -hairpin loop followed by two α -helix and the pre-S1-helix, which connects the ankyrin repeats to the first transmembrane segment [22,23]. The NH₂-terminal portion also contains a large number of cysteine and lysine residues that are important for forming disulfide bridges, which are critical for changing the structure upon receptor-agonist binding and are targets for electrophilic activators of TRPA1 [13]. TRPA1 also contains a calcium-binding EF-hand domain, commonly found in other calcium-interacting proteins [13]. Intracellular calcium ions can directly activate the channel, or potentiate agonist-induced responses, probably through this mechanism [13] (Figure 2).

2.2. TRPA1 localization

Initially, TRPA1 was described as a nociceptive channel with a plentiful presence in subpopulations of primary sensory neurons of the dorsal root (DRG), vagal (VG) and trigeminal (TG) ganglia, and responding to noxious compounds and low temperatures [11,24]. TRPA1 is mainly expressed in unmyelinated C-fibers and thinly myelinated A δ -fibers, and only occasionally large myelinated fibers [11]. The expression can vary between species. At least 25% of the TRPA1 expressing neurons are peptidergic, and therefore able to release substance P (SP) and the calcitonin gene-related peptide (CGRP). However, the colocalization of TRPA1 with non-peptidergic neuron markers, such as isolectin B4 (IB4), the purinergic P2X3 receptor, and the Na(V)1.8 channel, has also been observed [25–26–27]. TRPA1 is present in 30–50% of TRPV1-expressing neurons and rarely exists in neurons which do not express TRPV1 [28]. There is also evidence for a TRPV1-TRPA1 interaction, which suggests that the two proteins might form a heteromeric channel [29].

Although TRPA1 is mainly located in nociceptive neurons of the peripheral nervous system (PNS), it is also found at different sites of the central nervous system (CNS) [30]. Studies in rat brain have shown TRPA1 protein expression in the

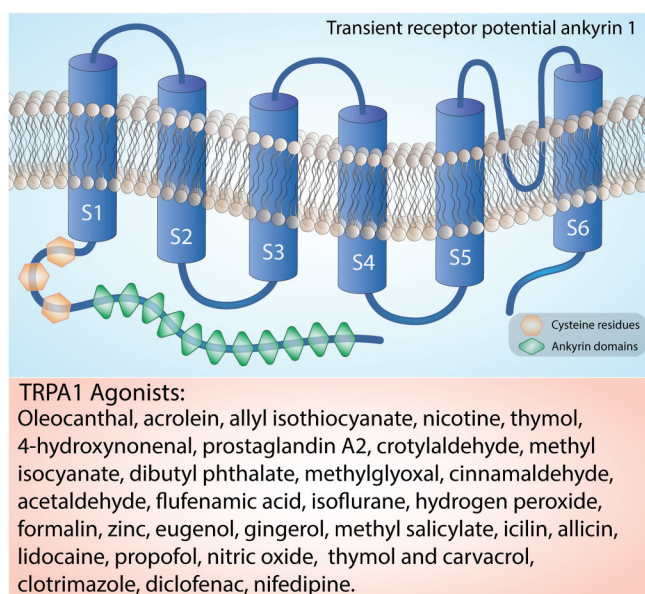


Figure 2. Structure of the TRPA1 channel. The TRPA1 channel is a homotetramer with each subunit containing six transmembrane helices, a series of ankyrin repeats, and intracellular NH₂- and COOH-termini. The transmembrane helices are labeled S1-S6. Orange box reports a list of the main TRPA1 agonists.

hippocampus, brainstem, olfactory bulb, striatum and amygdala [31-36-37]. In humans, TRPA1 has been detected in the cortex, caudate nucleus, putamen, globus pallidus, substantia nigra, hippocampus, cerebellum, amygdala, and hypothalamus [38]. However, the role of TRPA1 in the CNS is still unknown. TRPA1 expression has also been reported in chick [39], mouse, and human retina [40]. Emerging evidence has identified TRPA1 in a variety of additional extra-neuronal tissues where it contributes to different regulatory and proinflammatory pathways. These include the mouse inner ear and the organ of Corti [41], rat vascular endothelial cells [42], enterochromaffin cells of human and rat colon, cells of the respiratory tract [43,44], human keratinocytes and melanocytes, human synoviocytes, and human dental pulp and gingival fibroblasts [45]. The TRPA1 receptor can also be found in epithelial cells, mast cells, and pancreatic β cells [46-52-53]. More recent studies have reported the presence of TRPA1 in glial cells, such as astrocytes [54], oligodendrocytes [55] and Schwann cells [56,57]. It is worth noting that the presence of different TRPs, including TRPA1, has been observed in tumor tissues, including breast, pancreas, lung, liver, colon and gastroesophageal cancer, and has been proposed as a biomarker in cancer diagnosis and prognosis [58].

2.3. Agonists and modes of activation of TRPA1

TRPA1 agonists can be divided into two main groups: thiol-reactive electrophiles that modify the channel covalently, and compounds that modulate the channel differently. The first group includes a number of naturally occurring molecules often found in alimentary sources, including herbs and spices, such as allyl isothiocyanate (AITC) [14,59], allicin [24,60], and cinnamaldehyde [14]. Additional agonists characterized by remarkable chemical heterogeneity include volatile irritants,

such as acrolein and crotonaldehyde [61,62], chemicals of industrial origin [63-64-65], general anesthetics (e.g. isoflurane [66], lidocaine [67], propofol [68]), and laboratory chemicals (e.g. formalin [69-70-71]). Endogenous activators include oxidative stress components (e.g. nitric oxide [72], zinc [73,74], hydrogen peroxide [63-64-65], and methylglyoxal [22,75,76]). These TRPA1 agonists covalently modify the channel after association. A second group of TRPA1 agonists includes non-reactive compounds which are unable to modify the channel covalently and include plant origin, such as menthol [77], thymol and carvacrol [78,79]. Additional non-covalent activators are nicotine [80] and Δ^9 -tetrahydrocannabinol [59,81], and some common drugs, such as clotrimazole [82], nifedipine [83], and non-steroidal anti-inflammatory drugs, such as diclofenac [84] and acyl-glucuronide ibuprofen [85].

In addition, agonists of GPCRs, such as prostaglandins, bradykinin, histamine, and trypsin can induce intracellular changes that directly or indirectly result in TRPA1 activation [13]. TRP channel targeting stimulates calcium-dependent pathways, as these receptors allow the divalent cation influx from the extracellular space [2]. On the other hand, activation of phospholipase C (PLC) coupled receptors stimulates TRP channels, including TRPA1 [86]. Intracellular calcium, released by the endoplasmic reticulum, is an essential TRPA1 co-activator [59,87-90-91]. Intracellular pathways involving protein kinase A (PKA) have been shown to increase TRPA1 expression in the plasma membrane [92]. In particular, the receptor insertion on the membrane is regulated by PKA phosphorylation by increasing its expression in the lipidic layer [92]. Calcium release from intracellular stores and calcium influx from TRPV1 targeting also results in TRPA1 activation [13]. Ischemia usually increases proton concentrations, promoting TRPA1 activation and the ensuing increase in intracellular calcium concentration [55].

2.4. TRPA1 antagonists

The list of TRPA1 naturally occurring antagonists, which can be more appropriately labeled as partial agonists or weak inhibitors, includes camphor [93], camphor laurel, basil, wormwood, rosemary, 1,8-cineole, lemon eucalyptus, sage [5], and menthol, which, depending on the concentration, can either activate or inhibit the channel [77]. Gadolinium, amiloride, gentamicin and ruthenium red are nonselective TRPA1 antagonists [47].

The search for more selective TRPA1 channel antagonists has led to the development of molecules with optimization of existing scaffolds. Xanthine derivatives, including HC-030031, were the first identified as TRPA1 antagonists [71]. HC-030031 has proven effective *in vivo* at 100 and 300 mg/kg [71]. Later, other xanthinic derivatives were recognized as TRPA1 selective antagonists, including Chembridge-5861528, which is about 10 times more potent than HC-030031 [94,95]. Another antagonist is the oxime derivative, A-967079 (WO/2009/089082) [96]. A wide range of trichloro(sulfanyl)ethyl benzamides has also been produced and made available as potent TRPA1 antagonists, showing activity in human, but not in rat, TRPA1 [97]. More recently, additional TRPA1 antagonists have been reported. These include GRC 17536 [98], and in 2010,

a series of heterocyclic amides revealed properties of TRPA1 inhibition (2010/141805 A1) [99]. However, the promising compounds in this series suffer from poor solubility.

In 2011–2012, powerful antagonists of the TRPA1 channel were found among the derivatives of decalin (WO/2011/043954) and derivatives of proline (WO/2012/152983 A1) [100,101]. In 2013 and 2017, heterocyclic amides were also tested as TRPA1 antagonists (WO2013/108857 A1, WO/2017/135462 A1) [102]. Recently, some arylamide derivatives, carbamate compounds, azabenzofuran and 5-(2-(trifluoromethyl)phenyl)-indazoles, have been reported as TRPA1 antagonists with potency, metabolic stability, and considerable solubility [103,104]. In 2017, bicyclic heterocycle derivatives were patented as TRPA1 receptor antagonists (WO/2017/060488 A1). In 2018, the possibility of using ophthalmic preparations for the dispensing of eye drops for the treatment of eye diseases using TRPA1 antagonists was reported (WO/2018/009717 A1).

Among the various antagonists that have shown selectively toward TRPA1, only five have been tested in clinical trials for the treatment of pain or other conditions. These include: GRC 17536 (Glenmark Pharmaceuticals) [105], which, after a positive press release on its efficacy in painful diabetic neuropathy, has been the object of further publications; CB-189625 (Hydra Biosciences in partnership with Cubist Pharmaceuticals) was advanced into a phase-1 clinical trial for acute surgical pain [106], however, the study was discontinued due to disappointing pharmacokinetics; HX-100 (Hydra Biosciences) was tested for painful diabetic neuropathy and allergic asthma [107], however, no results have been reported so far; ODM-108 (Orion Pharma) was investigated for the treatment of neuropathic pain, however, the study failed, apparently for the poor pharmacodynamic features of the compound [108]; GDC-0334 (Genentech/Roche) which, developed for the treatment of asthma, has been tested in a phase-1 trial [109] but further development of the molecule was halted in 2019. A summary of clinical studies for TRPA1 antagonists is reported in Table 1.

3. Classifying pain

Pain may be classified according to different criteria based on the pathophysiological mechanism (nociceptive, inflammatory

or pathological pain) [110–112–113], duration (acute, chronic, episodic, or end of dose pain), etiology (malignant or nonmalignant) or the anatomical position [114]. Pain elicited by a physiological protective system from noxious stimuli is called nociceptive pain. Nociceptive pain is a short-lived condition generated by the body in response to a potentially harmful environmental stimulus; this generates reflexes that protect the individual from potential damage. Nociceptive pain is divided into two categories: somatic nociceptive pain, which is well localized usually at the level of the dermis, described as pungent, lacerating and burning, and visceral pain, which usually arises as a diffuse and poorly defined sensation perceived in the midline of the body, at the lower sternum, or upper abdomen [115].

Pain perception resulting from harmful cellular damage following surgical, traumatic, or disease-related injuries is defined as inflammatory pain. Inflammatory pain is adaptive and protective and is mainly caused by activation of the immune system following tissue injury or infection. In general, the intensity of pain is proportional to the extent of tissue damage and the release of inflammatory mediators, which are critical to initiate and sustain pain. Pathological pain does not occur as a symptom of a disorder, but rather represents a disease state of the nervous system, which can occur after damage to the nervous system (*i.e.* neuropathic pain) or in such conditions in which there is no specific damage or inflammation (*i.e.* dysfunctional pain). Acute pain has a short duration, is a consequence of tissue injuries, and tends to reduce in intensity over time. Chronic pain is more prolonged (it usually lasts at least 12 weeks), can be acute or dull, presents as a burning or pain sensation in the affected areas, can be present in all parts of the body, can be continuous or intermittent, can be difficult to diagnose, causes are not always clear, and it can greatly affect the patient's quality of life [116].

A variety of receptors in sensory neurons directly encode harmful stimuli that generate propagated action potentials to signal pain. These stimuli consist of physical (mechanical forces, temperature variations) and exogenous (irritants of vegetal origin such as capsaicin, menthol or isothiocyanates) and endogenous (protons, prostaglandins, kinins, and many others) chemical agents. Multi damage events may act

Table 1. Status summary of TRPA1 antagonists.

Compound	Company	Indication	Status	Side effects	References
GRC 17536	Glenmark Pharmaceuticals	Painful diabetic peripheral neuropathy, asthma	Discontinued	Not informed	[105]
CB-189625	Hydra Bioscience/Cubist Pharmaceuticals	Acute surgical pain	Discontinued for complex pharmacokinetics	Not informed	[106]
HX-100	Hydra Bioscience	Painful diabetic neuropathy, allergic asthma	Discontinued	Not informed	[107]
ODM-108	Orion Pharma	Neuropathic pain	Discontinued for complex pharmacokinetics	None	[108]
GDC-0334	Genentech/Roche	Asthma	Discontinued	Not informed	[109]

indirectly on the nociceptors, *via* the release of inflammatory substances and the accumulation of inflammatory cells, which favor nociceptor sensitization to any stimulus [117]. In this context, TRP channels have been identified as potential targets and multimodal sensors of irritative and pain signals. In addition, TRP channels may cooperate to encode a specific pain signal, as in the case of noxious heat, which requires the simultaneous contribution of TRPV1, TRPM3, and TRPA1 [118]. More importantly, robust evidence indicates TRPA1 as a major player in mediating the prolonged hypersensitivity to thermal, chemical, and mechanical stimuli detected in models of nociceptive, inflammatory and neuropathic pain [11,15,119-121-122].

4. TRPA1 and nociceptive pain

TRPA1 activators encompass an unprecedented series of inflammatory agents and mediators, including protons, nucleotides and nucleosides, enzymes (proteases), fatty acid derivatives (prostaglandins), biogenic amines (histamine, norepinephrine and serotonin), cytokines, chemokines, neurotrophins, and other peptides (bradykinin, endothelin). Nociceptors have an intrinsic activation threshold, and normally harmful stimuli of either mechanical, thermal or chemical origin must overcome this threshold to initiate action potentials to signal pain [117]. The involvement of TRPA1 in inflammatory hypersensitivity was initially suggested by the observation that TRPA1, *via* a PLC/calcium signaling pathway, contributes to bradykinin excitatory effects [14], thus representing an essential downstream target to induce nociceptor hypersensitivity [123].

The role of TRPA1 in inflammatory nociception has been described in a seminal work showing that either pharmacological blockade or gene deletion of the channel markedly reduced both the first and second phases of the nociceptive response induced by formalin in rat and mouse paw [71]. In addition to the acute nociceptive response, TRPA1 is involved in mechanical and thermal (cold) hypersensitivity, even days or weeks after the administration of the harmful stimulus, when damaging agents are removed and inflammation presumably resolved. Indeed, several studies have documented that acute pharmacological inhibition, using various TRPA1 inhibitors or TRPA1 gene deletion, reduced both the mechanical and cold hypersensitivity associated with a persistent inflammation in models of osteoarthritis, induced by complete Freund adjuvant, carrageenan, monosodium iodoacetate, and monosodium urate [124-131-132].

5. TRPA1 and neuropathic pain

Unlike inflammatory pain, neuropathic pain is not associated with an overt tissue inflammatory condition, but rather is caused by a lesion or dysfunction of the somatosensory system, including peripheral nerve fibers (A β , A δ and C fibers), and the ensuing altered transmission of sensory signals to the spinal cord and brain. Neuropathic pain affects 7–10% of the general population [133], and its incidence is likely to increase due to the rising age of the global population. Multiple causes of neuropathic pain have been described in the central and

peripheral nervous systems, including brain and spinal cord injury, neurodegenerative diseases, multiple sclerosis, diabetes, HIV infection, leprosy, chemotherapeutics, and alcohol abuse [134-141-142].

Several lines of evidence support TRPA1 implication in models of neuropathic pain, with the first report obtained by spinal nerve ligation in mice [143]. Downregulation of TRPA1 expression in L5/DRG, and upregulation in L4/DRG, suggested that a compensatory mechanism could occur after nerve injury. Subsequent studies showed a similar expression pattern using other nerve injury models, such as sciatic nerve injury by chronic constriction or transection [144-145-146]. All these data revealed a possible analgesic strategy by blocking/inhibiting TRPA1. Some TRPA1 antagonists given by different routes of administration have been used to reduce sensory hypersensitivity in rodent experimental models. Systemic blockade of TRPA1 decreased allodynia evoked by peripheral nerve injury [147], and intrathecal injection of TRPA1 antagonist attenuated the hypersensitivity in animals with a spinal nerve ligation model [148]. Further evidence has been obtained by genetic channel deletion [149,150], which inhibited mechanical allodynia [151], thus strengthening the hypothesis that TRPA1 plays a major role in mechanical hypersensitivity following nerve damage. In addition, TRPA1 inhibition may decrease postoperative pain, as the administration of a channel antagonist into the injured surgical area attenuated mechanical allodynia. Furthermore, an implication of CNS TRPA1 has been proposed, given that mechanical allodynia decreased after spinal administration of channel antagonists [94].

TRPA1 has been shown to contribute to peripheral neuropathic pain, not caused by mechanical trauma, such as the pain associated with diabetic neuropathy [95,152]. Peripheral neuropathy is a common characteristic complication of diabetes mellitus, thus causing cutaneous pain, often of burning quality. TRPA1 antagonism has been reported to reduce mechanical allodynia and hypersensitivity in a rodent model of streptozotocin-induced diabetes [153]. Furthermore, oxidative stress and glucose metabolism byproducts, such as 4-hydroxy-2-nonenal (4-HNE) and methylglyoxal, largely increased in diabetes, can activate TRPA1, thus contributing to hyperalgesia [22,154,155].

A common adverse-effect of anticancer drugs is a chemotherapeutic-induced peripheral neuropathy (CIPN), the symptoms of which usually encompass paresthesia and dysesthesia to the extremities, spontaneous pain, and mechanical and thermal hypersensitivity, provoking a prolonged and disabling condition [156]. TRPA1 is markedly implicated in mechanical and cold allodynia evoked in mouse models of CIPN and therefore represents a potential therapeutic target for the human condition. Anticancer drugs that have been reported to directly target TRPA1 to elicit mechanical and thermal hypersensitivity or nociceptive responses are oxaliplatin, paclitaxel, bortezomib, and the aromatase inhibitors exemestane, letrozole and anastrozole [127,157,158].

6. TRPA1 and cancer pain

Pain is a common and devastating symptom of cancer, which often impairs the quality of life more than cancer itself, and

affects about 70–90% of cancer patients [159]. As the cancer grows, tumor microenvironment composed by cancer cells, inflammatory and immune cells, and structural cells, progressively infiltrates the neighboring tissues and sends specific chemical signals, thus contributing to the development of a proalgesic neural environment [160,161]. Remodeling of the sensory fibers has been speculated to contribute to the generation of the spontaneous breakthrough episodes often observed in cancer patients, as similar neuroma-like structures have been reported in conditions characterized by spontaneous ectopic pain episodes, such as complex regional pain syndrome [162,163]. A very recent observation showed that oxidative stress and TRPA1 are essential in mediating mechanical and cold allodynia in a mouse model of metastatic cancer pain evoked by injection of melanoma cells in the hind paw as genetic deletion/pharmacological antagonism of TRPA1 or antioxidants attenuated pain-like responses [164].

7. TRPA1 and dysfunctional pain

Dysfunctional pain, which falls into the category of chronic pain, has been proposed recently (2010) [113,163–164–165]. Several clinical disorders have been associated with the onset of pain defined as dysfunctional, including primary headaches, fibromyalgia, temporomandibular disorder, back pain, interstitial cystitis, pelvic pain, and irritable bowel syndrome. Compared to other categories of pain, dysfunctional pain does not seem to have a well-defined cause, thus complicating diagnosis and treatment. One common feature of dysfunctional pain is the presence of unexplained pain as the main symptom which, however, significantly reduces the patient's quality of life. Currently available pharmacological treatments for dysfunctional pain conditions are often not satisfactory, thus highlighting the need for the discovery of new therapeutic targets.

Several lines of evidence support the implication of TRPA1 in models of conditions characterized by dysfunctional pain. Pharmacological inhibition of the TRPA1 channel or its deletion from primary sensory neurons have been shown to switch off periorbital mechanical allodynia in a mouse model of migraine induced by glyceryl trinitrate administration [166]. In a model of temporomandibular disorder induced by masseter muscle inflammation, TRPA1 and TRPV1 inhibition was reported to mitigate spontaneous pain [167]. Increased levels of methylglyoxal were found in the plasma of patients suffering from low back pain (LBP). In a rat model of LBP, methylglyoxal accumulation was reported in dorsal root ganglia [168], and TRPA1 can be implicated in this model as methylglyoxal is a channel agonist [75].

Epigenetic regulation of TRPA1 [169] seems to contribute to mechanical pain sensitivities in some multisomatoform disorders, including fibromyalgia, which is characterized by distressing and functionally disabling somatic symptoms with chronic pain as the most frequent and clinically relevant complaint. The gastrointestinal tract is among the most studied internal organs with regards to the role of TRPA1 in visceral inflammation and nociception. TRPA1 was found to mediate mechanical hypersensitivity to colonic distension in chemically induced colitis [170,171]. The dextran sulfate

sodium (DSS)-evoked inflammatory bowel disease in rodents has been associated with sensitization of colonic TRPA1, which, promoting the release of SP, sustains inflammation [172]. More recently, TRPA1 implication has been reported in the somatic mechanical hypersensitivity associated with DSS-induced colitis [173]. Furthermore, histamine sensitizes TRPA1 and TRPV4, thus promoting visceral pain in patients suffering from irritable bowel syndrome [174]. TRPA1 implication in the visceral pain associated to inflammatory conditions may suggest a major role for the channel in irritable bowel disease, thus representing an important candidate for the development of new treatments for this condition.

8. TRPA1 and migraine pain

Migraine pain is a major medical concern, due to the large prevalence of the disease in the general population. Although the underlying pathway responsible for migraine pain remains unknown, the introduction of small molecules that antagonize the CGRP receptor (CGRP-R) and monoclonal antibodies against CGRP or CGRP-R in acute and prophylactic migraine treatment indicates that the neuropeptide plays a major role in this disease [175,176]. The component of neurogenic inflammation produced by CGRP released from the terminals of trigeminal neurons seems to be one of the main mechanisms of migraine headaches. As TRPA1 stimulation may cause CGRP release, many migraine triggers activate TRPA1, and some drugs already used for migraine treatment can desensitize or inhibit TRPA1 [177], the channel may be considered a therapeutic target for migraine. Exposure to environmental and smoke irritants, such as acrolein and other chemicals known as TRPA1 agonists, elicits CGRP release and neurogenic inflammation in cranial districts [178]. All these effects can be inhibited by CGRP or TRPA1 antagonists [178]. Glyceryl trinitrate, which is known to elicit migraine attacks in patients [179] releases nitric oxide (NO), which induces pain-like responses *via* TRPA1 [72]. Glyceryl trinitrate elicits periorbital allodynia *via* TRPA1 NOX1/2 activation within the soma of trigeminal nociceptors and the ensuing ROS and CGRP release [166]. In addition, hydrogen sulfide, another gaseous stimulant of TRPA1, might contribute to the mechanism of migraine [180].

9. Glial cell TRPA1 and pain

While there is broad evidence that direct TRPA1 stimulation in nociceptors mediates acute pain responses, recent findings have shifted the focus to the TRPA1 expressed in glial cells as the critical factor to sustain chronic pain. It has long been known that neuropathic pain caused by nerve injury (Wallerian degeneration) is associated with a robust infiltration of macrophages within the damaged nerve trunk [56,181–182–183]. Findings obtained in a mouse model of type-II trigeminal neuralgia produced by infraorbital nerve constriction [183] have proposed that nociceptor TRPA1, targeted by the oxidative burst generated invading macrophages, conveys the pain signal to the brain. However, a subsequent study in a different mouse model of neuropathic pain (partial sciatic nerve ligation) provided evidence

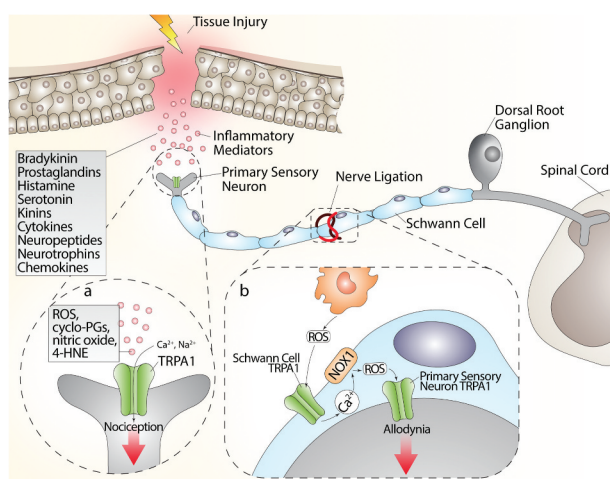


Figure 3. (a) Tissue injury generates a series of inflammatory agents, including reactive oxygen (ROS), nitrogen (nitric oxide) and carbonylic (4-hydroxy-2-nonenal, 4-HNE) species, bradykinin, prostaglandins, histamine, serotonin, kinins, cytokines, neuropeptides, and neurotrophins and chemokines. Some of these agents, such as ROS, cyclopentenone-prostaglandins (cyclo-PGs), nitric oxide, and 4-HNE, directly gate the channel, whereas other agents indirectly modulate TRPA1 activity, thus promoting intracellular signaling cascades. Activation of both pathways contributes to the generation of acute pain. (b) The injured nerve trunk releases proinflammatory chemokines, which recruit activated macrophages within the lesioned area. Phagocyte-dependent oxidative stress (ROS) activates TRPA1 in Schwann cells, which evokes a calcium (Ca^{2+})-dependent, NADPH oxidase 1 (NOX1)-mediated amplification of hydrogen peroxide (H_2O_2) release, which targets nociceptor TRPA1 to signal mechanical allodynia.

that macrophage-dependent oxidative stress does not directly target TRPA1 in nociceptors, but rather activates the channel in Schwann cells that ensheath the nerve fibers [56]. Then, *via* an NADPH oxidase 1 (NOX1)-dependent pathway, Schwann cell TRPA1 amplifies and sustains the oxidative stress signal, which exerts a dual function. An outwardly directed release of oxidants maintains macrophage recruitment inside the injured nerve trunk, and an inwardly directed oxidative stress targets nociceptor TRPA1 to signal pain [56] (Figure 3).

However, the contribution of activated macrophages is not necessarily required to activate the proalgesic Schwann cell TRPA1 pathway. In fact, in a model of alcohol-evoked painful peripheral polyneuropathy [57], we found that oral ethanol is converted by Schwann cell alcohol dehydrogenase-2 (ADH-2) into acetaldehyde, which, in an autocrine manner, targets Schwann cell TRPA1 to generate ROS and 4-HNE, which in turn activates TRPA1 in nociceptors to signal pain [57]. In this case, macrophage contribution is not required, given that Schwann cells, *via* their own enzymatic machinery (ADH-2), generate the oxidative stress burst necessary to act on TRPA1 to sustain neuroinflammation.

10. Conclusions

Chronic pain is a major health problem, which leads to a decrease in the quality of life for many people worldwide. Strong efforts are being made toward limiting opiate use and pursuing safer treatments. Given its expression in many different types of tissues and cells, and its pleiotropic biological

profile, TRPA1 represents an attractive therapeutic target in a variety of human diseases. Robust preclinical evidence accumulated in the last 20 years indicates TRPA1 as a potential target for the treatment of pain. Recent investigations have underlined the crucial role of the feed-forward mechanism that encompasses Schwann cell TRPA1 and oxidative stress in peripheral nerve fibers to sustain mechanical allodynia. These novel findings open new potential strategies for the treatment of chronic pain.

11. Expert opinion

Tissue insult may generate an acute painful effect, associated with early neural and cellular responses aimed at removing harmful stimuli, thereby limiting further tissue injury, and favoring repair. Response to tissue injury may evolve into a chronic status of allodynia and hyperalgesia, typically represented by hypersensitivity to both mechanical and thermal stimuli. In addition to central mechanisms, this hypersensitivity is also driven by over-activity of the peripheral nociceptors *via* sensitization of their nerve terminals. Several different etiologic agents, including physical trauma, neurotoxins, cancer and chemotherapeutic treatment, infections, and immune and metabolic diseases, produce pain symptoms, in which different molecular mechanisms contribute to the sensitization of small-diameter unmyelinated C-fibers and medium-diameter thinly myelinated A δ -fibers. Although large advances have been made in pain research during the past decade, there has been little translation of preclinical results into clinical practice. Currently, very few novel therapeutic opportunities have been offered to patients, and older drugs have considerable side effects and incomplete efficacy. For these reasons, patients are frequently undertreated, and new, safer and more effective analgesic drugs are clearly needed.

TRPA1, also known as the proalgesic wasabi receptor, belongs to the larger TRP family of channels, which regulates numerous homeostatic functions and pathological processes. TRPA1, while maintaining a conserved sensitivity in chemosensation across species, shows remarkable variability regarding cold and mechanical sensitivity.

Localization of TRPA1 in a large variety of cells and tissues suggests pleiotropic roles. Since its identification in C-fibers [11] more than 10 years ago, the TRPA1 channel has been deeply scrutinized as a pain transduction mechanism. Robust evidence implicates TRPA1 in pain processing at different anatomical sites of nociceptors, from peripheral terminals and DRG somata, to central terminals in the spinal cord. Sensory neuron TRPA1, gated by an unprecedented variety of exogenous stimuli and endogenous mediators, is primarily activated by oxidative stress byproducts, which are abundantly generated at sites of tissue injury and inflammation. Because of this wide range of agonists, TRPA1 has been proposed to markedly contribute to acute nociception [63,71,124-131-132]. In addition, due to its prominent localization to peptidergic primary sensory neurons [11] TRPA1 activation results in the release of SP and CGRP from peripheral terminals, thus mediating neurogenic inflammatory responses [166,184]. The release of CGRP from terminals of peptidergic nociceptors is now recognized as a major mechanism of migraine pain [175]. Thus, TRPA1 represents an excellent

candidate for analgesic drug design to treat acute pain of different origins and migraine headaches.

However, recent findings in murine models of neuropathic pain have shifted the focus from neuronal to glial TRPA1. TRPA1 expressed in Schwann cells has been found to dynamically communicate with the ensheathed nerve fibers and with surrounding inflammatory cells (expanded peripheral nerve resident macrophages or invading hematogenic macrophages) *via* oxidative stress generation. The feed-forward mechanism that encompasses invading macrophages, oxidative stress, and Schwann cell TRPA1 was found to be essential to sustain mechanical allodynia in mouse models of neuropathic pain [56], and of complex regional pain syndrome type I [185]. In addition, Schwann cell TRPA1 has been reported to generate, in a macrophage-independent manner, oxidative stress, which sustains mechanical allodynia in a chronic murine model of alcoholic neuropathy [57]. Thus, the possibility to selectively target the channel in Schwann cells, may provide a unique opportunity to selectively switch off chronic hypersensitivity.

With the caveat that most relevant models are reproduced in rodents, TRPA1 contribution to inflammatory and neuropathic pain is robustly emerging. The pronounced sensitivity of TRPA1 to oxidative stress and its byproducts emphasizes the channel's role in mediating responses evoked by such mediators at sites of tissues injury and inflammation. During the last five years, significant progress has been made toward the discovery of new TRPA1 antagonists, although drug development programs are still limited, and initial promising results with TRPA1 antagonists, which are under current clinical scrutiny, require further confirmation before they can enter the therapeutic armamentarium.

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Declarations of interest

F De Logu, P Geppetti and R.Nassini are founding scientists of FloNext Srl. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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