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Transient Receptor Potential Vanilloid 4: The Sixth Sense of the Musculoskeletal System?

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Abstract

The critical discovery in the past two decades of the Transient Receptor Potential (TRP) superfamily of ion channels has revealed the potential mechanisms by which cells sense diverse stimuli beyond the prototypical “five senses”, identifying ion channels that are gated by heat, cold, mechanical loading, osmolarity, and other physical and chemical stimuli. Transient receptor potential vanilloid 4 (TRPV4) is a Ca²⁺-permeable non-selective cation channel that appears to play a mechano- or osmosensory roles in several musculoskeletal tissues. In articular cartilage, TRPV4 exhibits osmotic sensitivity, controlling cellular volume recovery and other physiologic responses to osmotic stress. TRPV4 is expressed in both osteoblasts and osteoclasts, and the absence of TRPV4 prevents disuse-induced bone loss. TRPV4 activation promotes chondrogenesis by inducing SOX9 transcription, whereas a TRPV4 gain-of-function mutation leads to a developmental skeletal dysplasia, suggesting a critical role for TRPV4 in skeletal development. These studies provide mounting evidence for a regulatory role for the sensory channel TRPV4 in control of musculoskeletal tissues.

Keywords

osteoarthritis; osteoporosis; stretch-activated ion channel; mechanotransduction; mechanical signal transduction; remodeling

The Transient Receptor Potential Superfamily of Ion Channels

The discovery of the Transient Receptor Potential (TRP) superfamily of cation channels has begun to reveal the mechanisms by which cells are able to sense a diverse array of stimuli, such as heat, cold, mechanical loading, and osmolarity, playing vital roles in the normal function of the body. These channels, which are divided into seven subfamilies by sequence homology (TRPC, TRPV, TRPM, TRPN, TRPA, TRPP and TRPML) are generally activated by chemical agonists as well, and in many cases, are believed to serve as “integrators” of physical and chemical stimulants. For example, TRPV1 is well known as the “chili-pepper” receptor and is activated chemically by capsaicin, whereas its function in vivo appears to be that of a noxious heat and pain receptor.¹ TRPM8 serves as the body’s “cold receptor”, and is activated chemically by menthol.²

TRP ion channels were first described in 1989 in *Drosophila* as a result of molecular cloning of the *transient receptor potential* phototransduction mutant.³ This invertebrate-vertebrate gene family comprises >60 members (28 in mammals). Within the TRP superfamily of ion

channels, the TRPV subfamily came into the spotlight in 1997,^{1, 4} with the discovery of its initial members, TRPV1 in mammals and OSM-9 in *Caenorhabditis elegans*.

Of the TRP superfamily, several channels appear to regulate the body's local as well as systemic responses to mechanical and osmotic stimuli, as part of a functional group termed "osmo-mechano-TRPs", including TRPV1, TRPV2, TRPV4, TRPA1, TRPM3, TRPM7, TRPC1, TRPP2, TRPML3, and the invertebrate channels OSM-9, OCR-2, NAN, NompC, and IAV.⁵ In this respect, the discovery of these channels has provided new insights into the mechanosensory capabilities of non-sensory cells of the musculoskeletal tissues such as cartilage, bone, and muscle. These musculoskeletal tissues show exquisite sensitivity to their mechanical environment, and it is well recognized that mechanical stresses acting at the cellular level are important signals controlling the physiology as well as pathology of such tissues. In particular, TRPV4 appears to play important roles in several regulatory pathways involved in skeletal development or homeostasis. This review focuses on the potential role of TRPV4 as sensory ion channel in musculoskeletal tissues that may serve as an integrator of physical and chemical signals.

The Mechano-osmosensitive Properties of TRPV4

Evidence supporting the notion of osmo-sensitivity of TRPV4 was provided by the observation that CHO cells, which do not normally express TRPV4, responded to hypo-osmotic stress when transfected with TRPV4.⁶ In other studies, expression of TRPV4 in batches of HEK 293T cells that normally did not express TRPV4 showed the sensitivity of TRPV4 was also modulated by temperature.⁷ Interestingly, these cells showed no response to mechanical stretch at room temperature (under iso-osmotic conditions), but at 37°C, stretch induced a maximal Ca²⁺ influx compared to all conditions. Similarly, other studies have shown that TRPV4 osmotic responsiveness is temperature-dependent,⁶ although it has been experimentally difficult to strictly separate mechanical and thermal effects.⁸ Importantly, the specific pathways involved in the transduction of the TRPV4-mediated signal appear to be specific to the different types of stimuli (e.g., tonicity, temperature, or chemical agonists).⁹

Systemic osmolarity is one of the most tightly regulated physiologic parameters.¹⁰ In response to systemic hyper-osmolarity, *Trpv4*^{-/-} mice are unable to regulate tonicity as efficiently as wild-type control mice.¹¹ Interestingly, these knockout mice showed reduced drinking, either spontaneously or in response to intraperitoneal application of hypertonic saline, and their levels of antidiuretic hormone secretion in response to osmotic stimulation was also reduced. These mice also showed impaired osmotic activation, as systemic hyper-osmotic stress led to reduced expression of the immediate-early transcription factor c-FOS in the nuclei of cells located in the circumventricular organ, the organum vasculosum of lamina terminalis (OVLT). These findings indicate a deficit in central osmotic sensing in *Trpv4*^{-/-} mice, suggesting that this channel is necessary for systemic homeostasis of mammalian osmolarity. *Trpv4*^{-/-} mice also show altered responses to mechanical stimulation,^{11, 12} and the absence of TRPV4 has been associated with an elevation in the threshold for noxious mechanical stimuli.

The case for TRPV4 playing a role in human osmoregulation has recently been augmented. A recent study has shown that an N-terminal point mutation in the human *TRPV4* gene, TRPV4_{P19S}, was associated with increased prevalence of hyponatremia in certain populations.¹³ Interestingly, this mutation diminished the response of TRPV4 to moderate hypotonic stress (15%) and to the lipid epoxyeicosatrienoic acid, but not to 4αPDD or higher hypotonic stress (30%), suggesting that the mutation possibly does not completely abolish TRPV4 function, but renders it less sensitive.

The role of TRPV4 as a mechano-osmosensor has been clearly demonstrated through its ability to restore the osmotic avoidance behavior of *Caenorhabditis elegans*. Using a forward genetics screen in *C. elegans* with a high molarity osmotic barrier, it was found that *osm-9* mutant worms, unable to sense the osmotic barrier, harbored a mutation in a TRP channel gene.⁴ However, the expression of mammalian TRPV4 in the sensory ASH neuron of *osm-9* mutants was able to rescue the defective avoidance of hyper-osmotic stimuli and nose-touch avoidance,¹⁴ despite the < 26% orthology between OSM-9 and TRPV4 proteins.

Whereas TRPV4 was originally identified as an osmotically activated channel,^{6, 15} evidence gathered since its discovery years ago illustrates that the channel can also be activated/modulated by a diverse range of factors such as mechanical stimuli, heat, possibly low pH, and eicosanoids (e.g.,^{5, 16–18}). However, robust activation of TRPV4 by hypotonicity and mechanical stimulation has been demonstrated specifically by several groups independently and convincingly in a number of mammalian cells and organ systems, with evidence for a critical role of this channel in cell volume regulation, response to shear stress, as well as other forms of mechanotransduction (e.g.,¹⁹).

TRPV4 in the Skeletal System

The mechanosensitivity of a wide variety of musculoskeletal tissues has been demonstrated through numerous in vivo and in vitro studies. For example, the remodeling response of bone to mechanical loading is well recognized, although the cellular sensors involved in signal transduction have remained elusive.²⁰ Similarly, joint loading is a critical signal in the maintenance of cartilage homeostasis, yet abnormal mechanical loading due to joint instability, trauma, or obesity is a primary risk factor for osteoarthritis.²¹

In particular, cells in cartilaginous tissues such as articular cartilage and intervertebral disc are normally exposed to dynamic changes in their mechanical and osmotic environments, and these signals appear to play a significant regulatory influence in tissue maintenance.^{22, 23} The transduction of mechanical loading in the cartilage extracellular matrix appears to involve intermediary changes in other biophysical parameters, particularly physicochemical effects such as osmotic stress. Due to the large concentration of proteoglycans bound within the cartilage matrix, chondrocytes experience large fluctuations in their osmotic environment due to local changes in the interstitial water content as a function of loading and unloading of the joint. Previous studies have shown that chondrocytes respond directly to mechanical and osmotic stresses with volumetric changes that are associated with increases in the intracellular concentration of Ca^{2+} ,^{24–26} followed by regulatory volume recovery that involves remodeling of the F-actin cytoskeleton²⁵ and/or the transport of solutes and ions across the cell membrane.^{27–29}

Recent studies have provided compelling evidence that the response of chondrocytes to hypo-osmotic stress is regulated primarily by TRPV4.³⁰ Articular cartilage exhibits abundant expression of TRPV4 at the RNA and protein levels, and chondrocytes show high sensitivity to the TRPV4-selective activator, 4aPDD. The inhibition of TRPV4 using a specific antagonist, GSK205, has been shown to diminish chondrocyte response to osmotic stress, reducing the fraction of Ca^{2+} responsive cells, the extent of regulatory volume decrease, and the production of prostaglandin E2 caused by cell swelling. Interestingly, the specific activation of TRPV4 was able to restore defective regulatory volume decrease that is caused by the pro-inflammatory mediator, interleukin 1. While TRPV4 appears to be present throughout the chondrocyte membrane, chemical disruption of the primary cilium using chloral hydrate was found to eliminate osmotically- or 4aPDD-induced Ca^{2+} signaling.³⁰ These findings indicate that TRPV4 is present and functional as an osmosensor

in articular chondrocytes, potentially modulating the production or influence of pro-inflammatory molecules in response to osmotic stress.

Recently, in zebrafish, a *Trpv4*-related cDNA was isolated, and its sequence and expression profile render it an attractive candidate to be zebrafish TRPV4, with 84% amino-acid homology to human.³¹ By *in situ* hybridization, it was expressed in mechanosensitive hair cells, in endothelia, and renal epithelia, just like its mammalian counterpart. With relevance to this overview, it was found expressed first in the developing notochord, then later in chondroblasts of developing pectoral fins and jaws. Importantly, these findings suggest that TRPV4 expression in chondrocytic lineages has been conserved throughout vertebrate evolution.

In other studies, a functional gene screen was performed to determine which chondrocyte genes participate in activation of Sox9, a transcription factor that functions as chondrogenesis master regulator.³² The surprising finding of this study was that TRPV4 activation was responsible for promoting chondrogenesis in chondrogenic (ATDC5) and mesenchymal (C3H10T1/2) mouse cell lines, by inducing SOX9 transcription through a Ca^{2+} /calmodulin pathway. Furthermore, *Trpv4* mRNA was found to be present in chondrocytes from rib and articular cartilage of mice. Subsequently, global transcriptome analysis has revealed the differential expression of *Trpv4* in the pre-condensed mesenchyme of mouse hind limbs, which was verified by quantitative PCR and *in situ* hybridization,³³ supporting the potential role of this channel in chondrogenesis as part of the development and maturation of the skeleton.

In addition to its role directly in cartilage, functional TRPV4 has also been identified in synoviocytes,^{34–36} which play a significant role in the inflammatory component of arthritis. TRPV4 is robustly expressed in synoviocytes from both normal and rheumatoid arthritis afflicted patients. It is, however, largely absent from the synoviocyte cell line MH7A, but present in the SW982 synoviocyte cell line.^{34, 35} Similar to chondrocytes,³⁰ activation of synoviocyte TRPV4 with 4aPDD has been shown to decrease the response to the pro-inflammatory cytokine interleukin 1. Furthermore, treatment of synoviocytes with another pro-inflammatory mediator, tumor necrosis factor- α , has been shown to cause increased TRPV4 gene and protein expression, as well as increased TRPV4-mediated sensitivity to hypo-osmotic stress.³⁶

The critical role of TRPV4 in skeletal development was demonstrated through the discovery that a TRPV4 gain-of-function mutation was found associated with a familial form of brachyolmia, a developmental skeletal dysplasia.³⁷ In this study, the human mutated forms of TRPV4 were expressed in HEK cells to examine their electrophysiologic properties. In comparison to the wild-type channel, the human mutations yielded increased constitutive levels of activation, with markedly increased maximal 4aPDD-activated current. However, the injury mechanisms and relevant skeletal cell type remain incompletely understood in this novel TRPV4-channelopathy.

In addition to its important role in chondrogenesis and mechano-osmoregulation of cartilage, TRPV4 also appears to have a critical influence on homeostasis of the bony skeleton. Recent studies have identified the presence of numerous TRP channels, including TRPV4, in osteoblasts osteoblast-like cells.^{38, 39} Importantly, however, the deletion of TRPV4 has been shown to increase bone mass in *Trpv4*^{-/-} mice by impairing bone resorption through the modulation of osteoclast differentiation.⁴⁰ In this study, TRPV4-mediated Ca^{2+} oscillations were necessary for the control NFATc1-regulated gene transcription, and thus the terminal differentiation and activity of osteoclasts. Similarly, *Trpv4*^{-/-} mice exhibit suppression of bone loss following unloading of the skeleton, as evidenced by less reduction in the levels of

mineral apposition rate and bone formation rate as compared to wild-type mice.³⁹ Furthermore, *Trpv4*^{-/-} mice exhibited a decreased number of osteoclasts in the primary trabecular bone following unloading.

Conclusions

Taken together, these in vitro and in vivo studies provide compelling evidence of the critical role for TRPV4 in the development and maintenance of skeletal tissues. Several TRP channels, particularly TRPV4, exhibit mechano- and osmosensitivity, thus providing highly promising candidates for the elusive “mechanosensors” responsible for controlling cellular response to loading in musculoskeletal tissues such as cartilage and bone in concert with inflammatory and pro-degenerative chemical signals in the microenvironment in pathological circumstances. Further investigation on the expression and function of this channel superfamily will provide exciting new insights in the regulation of skeletal development, physiology, and disease.

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