Rare Diseases 2:1, e962971; September 1, 2014; © 2014 Taylor & Francis Group, LLC

Unraveling the mechanism by which TRPV4 mutations cause skeletal dysplasias

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Transient Receptor Potential Vanilloid 4 (TRPV4) is a mechano- and osmosensitive cation channel that is highly expressed in chondrocytes, the cells in cartilage. A large number of mutations in TRPV4 have been linked to skeletal dysplasias, and the goal of this addendum is to shed light on the mechanisms by which mutations in TRPV4 can cause skeletal dysplasias by focusing on 3 recent publications. These papers suggest that skeletal dysplasia-causing TRPV4 mutations reprogram chondrocytes to increase follistatin production, which inhibits BMP signaling, thus slowing the process of endochondral ossification and leading to skeletal dysplasia. In spite of these important advances in our understanding of the disease mechanism, much remains to be elucidated. Nonetheless, these new data suggest that inhibiting aberrant TRPV4 activity in the cartilage may be a promising direction for therapeutic intervention.

Keywords: arthropathy, arthritis, bone morphogenetic protein, brachyolmia, cartilage, calcium signaling, growth plate, osteoarthritis, skeletal dysplasia

Abbreviations: TRPV4, transient receptor potential vanilloid 4; BMP, bone morphogenetic protein; TRP, transient receptor potential; TRPV, transient receptor potential vanilloid; SRY, sex determining region Y; SOX9, SRY-box 9; Col2a1, collagen type II, alpha 1; iPSCs, induced pluripotent stem cells; RUNX2, runt-related transcription factor 2; FST, follistatin; BV/TV, bone volume per trabecular volume; CRE, cyclic AMP response element; CREB, CRE binding protein

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Submitted: 06/05/2014

Accepted: 09/03/2014

http://dx.doi.org/10.4161/2167549X.2014.962971

Introduction

The Transient Receptor Potential (TRP) superfamily of ion channels has been implicated in sensation of a variety of physical and chemical stimuli throughout the body. TRP channels are typically non-selective cation channels that preferentially permeate calcium due to cellular calcium gradients.[1] Most TRP channels contain 6 transmembrane domains with cytoplasmic termini that may interact with other proteins and the cytoskeleton. The TRP Vanilloid (TRPV) channels have been implicated in thermo-, osmo-, and mechanosensation in a wide variety of tissues. In particular, TRPV4 is a polymodally activated TRP channel that is expressed in a variety of tissues and exhibits diverse functions. In sensory neurons, such as the dorsal root and trigeminal ganglion neurons, TRPV4 has been shown to play a role in mechanosensation, osmosensation and nociception/pain.[2,3] TRPV4 activation in keratinocytes of the skin by UVB mediates sunburn pain and tissue damage.[4] TRPV4 is also expressed in kidney, where it acts not only as an osmosensor, but also as a mechanosensor that can sense shear flow.[5] Furthermore, certain TRPV4 polymorphisms in humans coincide with alterations in systemic osmolarity.[6] In knockout mice, the lack of TRPV4 decreases homeostatic water intake behavior and thus increases systemic osmolarity.[7] In zebrafish, in situ hybridization has shown high TRPV4 expression in the developing kidney and in developing cartilage.[8]

TRPV4 in the Skeletal System

Along with diverse roles throughout the body, TRPV4 plays a crucial role in the skeletal system, where it is expressed in several tissues, such as cartilage and bone.[9] In bone, TRPV4 plays a key role in bone homeostasis by regulating osteoclast differentiation. Mice lacking TRPV4 have greater bone mass due to impaired bone resorption.[10] In cartilage, TRPV4 has been shown to play a crucial role in chondrocyte mechanotransduction and osmosensation.[11,12] Blocking TRPV4 in chondrocytes prevents an anabolic response to mechanical loads, while activating TRPV4 mimics the mechanical

loading response.[12] In cartilage, osmotic fluctuations occur as the tissue is mechanically loaded, and chondrocytes sense hypoosmotic change via TRPV4.[11] Furthermore, TRPV4 is involved in the normal maintenance of the joint, as mice lacking this channel develop earlier and more severe osteoarthritis than their wild-type counterparts.[13] Not only does TRPV4 play a key role in adult chondrocytes, it is also involved in differentiation of stem cells into a chondrogenic lineage.[14,15] TRPV4 expression levels increase as ATDC5 and C3H10T1/2 cells differentiate into chondrocytes. Furthermore, activation of TRPV4 in these cells, in concert with growth factors, leads to increased levels of the chondrogenic transcription factor, SRY (sex determining region Y)-box 9 (SOX9), and synthesis of cartilage-specific matrix molecules.[15] TRPV4 may also be involved in the conversion of cartilage to bone as it plays a role in chondrocyte volume regulation, which is integral to chondrocyte hypertrophy during endochondral ossification.[16]

TRPV4 Mutations

The first disease-causing TRPV4 mutations were identified in 2008.[17] Since that time, TRPV4 mutations that cause diseases, i.e., "channelopathies," have been identified at an astonishing rate, including 47 skeletal dysplasia-inducing mutations[18], 20 neuropathy-inducing mutations[19], and one osmoregulatory polymorphism.[6] The skeletal dysplasia mutations have a wide range of functional consequences, from relatively mild brachyolmia to severe neonatal lethal metatropic dysplasia.[20] Interestingly, these mutations occur at a wide range of sites throughout the TRPV4 protein, with no apparent relationship between mutation site and functional consequences: 4 mutations are in the amino-terminus, 26 are within the ankyrin repeat domains, 18 are within the transmembrane domains, and 9 are in the carboxy-terminus.[20] While some mutations cause both neural and skeletal disease,[21] it is surprising to find seemingly very different disease phenotypes caused by a single channel,[20] further supporting the diverse and tissue-specific function of this channel.

New Insight Into How TRPV4 Mutations Cause Skeletal Dysplasia

Our recent paper[22] and 2 other reports[23,24] have begun to unravel the mystery of how these TRPV4 mutations cause skeletal dysplasias. Briefly, Weinstein et al. describe the process of endochondral ossification that is affected by a TRPV4 mutation and leads to skeletal dysplasias.[23] Saitta et al. describe how the biochemical pathway driving endochondral ossification is malfunctioning.[24] Our study provides a mechanism that explains how the mutations are able to affect that pathway by overproducing an inhibitor of bone morphogenetic proteins (BMPs).[22] Importantly, given the tissue-specific function of this channel, these 3 studies have examined the effects of TRPV4 mutations in cartilage and chondrocytes, which provide an appropriate cellular context as the tissues and cells most affected by the mutations. Taken together, these studies provide new insights into how TRPV4 mutations can lead to skeletal dysplasias.

The earliest description of TRPV4 mutation-induced skeletal dysplasias speculated that these malformations might develop because of problems with endochondral ossification.[17] Further studies examined cartilage from individuals with severe lethal metatropic dysplasia and found poorly organized growth plate cartilage with few hypertrophic chondrocytes and islands of cartilage left in zones that should be fully mineralized, which also suggested problems with endochondral ossification of the cartilage.[25] Weinstein et al. created mice that expressed the $TRPV4_{R594H}$ mutation in order to examine more carefully how the mutation alters the process of endochondral ossification.[23] The channelopathy mutation was directed to chondrocytes by the collagen, type II, alpha 1 (Col2a1) promoter, an early and strong chondrocytic promoter. In humans, $TRPV4_{R594H}$ causes a moderately severe skeletal dysplasia, spondylometaphyseal dysplasia, Kozlowski type. The mice not only had major skeletal deformities, but also showed clear evidence of impaired differentiation of hypertrophic chondrocytes, with the most severely affected mice having almost no mineralization in endochondral bones. Taken together, these data suggest that robust expression of a skeletal dysplasia-causing TRPV4 channelopathy mutation, directed early to the chondrocytic lineage, evokes severe skeletal deformities that resulted from dysfunctional endochondral ossification.

With the knowledge that improper endochondral ossification is responsible for the skeletal deformities, the next question is what causes the problems with endochondral ossification. A recent paper by Saitta et al.[24] has very effectively shown how the endochondral ossification process is disrupted in the presence of a TRPV4 dysplasia-inducing mutation. Using induced pluripotent stem cells (iPSCs) derived from patients with the severe lethal $TRPV4_{I604M}$ mutation, they created stem cell based micromasses and examined their susceptibility to key regulators of chondrocyte hypertrophy. In normal cells, BMP2 drives the production of the key chondrocyte proteins type II collagen, SOX9, and aggrecan; and, during chondrocyte hypertrophy, it drives the production of type X collagen and runt-related transcription factor 2 (RUNX2). In iPSCs with the $TRPV4_{I604M}$ skeletal dysplasia mutation, mRNA for all of these genes was significantly decreased compared to micromasses from control iPSCs. The micromasses with the $TRPV4_{I604M}$ mutation were significantly less sensitive to BMP2.

This finding of decreased BMP2 sensitivity fits like the missing puzzle piece into our recent findings of how TRPV4 channelopathy mutations can cause skeletal dysplasias (**Fig. 1**). Our findings show that Ca^{2+} entering through the mutant TRPV4 channels activates a maladapted genetic program, which leads to the overproduction of follistatin (FST), a known and potent binding and neutralizing factor for BMP-related ligands. FST overproduction is transcriptionally mediated and critically depends on Ca^{2+} entering the chondrocyte

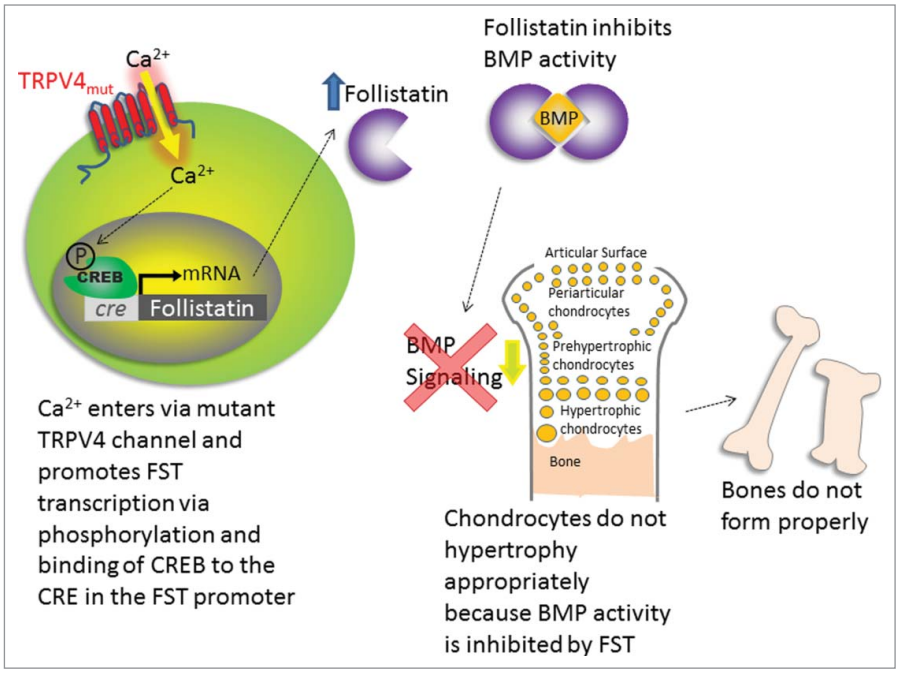

Figure 1. Schematic of the mechanisms by which TRPV4 mutations may lead to skeletal dysplasias. Ca^{2+} enters through the mutant channel, causing phosphorylation of CREB, which binds CRE and causes FST transcription. FST inhibits BMP activity, which prevents chondrocytes from undergoing hypertrophy and forming bone, thus leading to skeletal dysplasia.

via the TRPV4 channelopathy mutation.[22] Indeed, the expression of a variety of skeletal dysplasia-inducing TRPV4 mutations in chondrocytes results in overexpression of FST. This increase in FST expression is specific to skeletal dysplasia-inducing TRPV4 mutations, in that an arthropathy-inducing TRPV4 mutation does not cause an increase in FST. BMP signaling is key for the transition of chondrocytes to hypertrophic chondrocytes and onto bone,[26] and FST attenuates BMP signaling by acting as a "BMP-sponge," preventing BMPs from binding to their receptors. In separate experiments using a chick embryo model, we also confirmed that excess FST reduced bone ossification in developing limbs. Another piece of the puzzle worth mentioning dates back more than a decade to a study by Devlin et al., which showed that overexpression of noggin, an inhibitor of BMP, caused a significant decrease in bone volume per trabecular volume (BV/TV) (calcified bone fraction) in the mouse skeleton.[27] Similarly, we also saw a significant decrease in BV/TV in $TRPV4_{V620I}$ mice, which overexpressed FST. Therefore, with the overexpression of 2 different BMP antagonists, a similar reduction in bone calcification occurs.

The Complex Role of Calcium

Most of the TRPV4 channelopathy mutations that have been described result in an alteration in channel conductance, which results in altered levels of intracellular Ca^{2+}. Most of the dysplasia-causing mutations result in increased basal current density with both increased basal Ca^{2+} levels and higher Ca^{2+} levels in response to channel activation.[17,22,25,28,29] We and others have looked for correlations between these Ca^{2+} levels and disease severity, as it seems logical that, if altered channel function is causing disease, the more affected channel activity should cause worse disease. Surprisingly, there appears to be no direct correlation between the current density or Ca^{2+} concentrations and disease severity.[22,28] Loukin et al., however, did find a trend between the relative change in current in response to TRPV4 agonists and disease severity.[28] When averaged across broad disease categories, the relative agonist-induced current decreased as disease severity decreased, which may be due to an increase in basal current. However, many individual mutations deviated from this trend (which did not reach statistical significance), suggesting that this measure does not satisfactorily explain the relationship between channel activity and disease severity.

Thus, there appears to be a complex relationship between disease severity and Ca^{2+}. While we did not see a relationship between basal Ca^{2+} or agonist-induced peak Ca^{2+} with disease severity, we did find a Ca^{2+}-dependent mechanism that causes increases in FST, which could lead to skeletal dysplasia. This FST increase was prevented when the channel was mutated so that it could not permeate Ca^{2+}, either via a mutation that blocked the channel completely or a mutation that allowed monovalent, but not divalent cations to pass.[22] Antagonism of channel activity by a small molecule inhibitor also prevented the FST increase. Furthermore, the FST promoter contains a Cyclic AMP Response Element (CRE). This CRE is thought to be Ca^{2+} sensitive in that a Ca^{2+} signal can activate Ca^{2+}/calmodulin-activated kinase that phosphorylates CRE binding protein,[30] which then binds CRE and promotes FST transcription. Our results unambiguously demonstrate that the TRPV4 mutation-driven FST increase requires an intact CRE in the promoter of the *FST* gene.[22] Thus, FST upregulation depends on Ca^{2+} influx via TRPV4 channelopathy mutations. It also critically relies on an intact CRE DNA sequence in the *FST* promoter, a transcriptional activation mechanism that is known to be Ca^{2+}-dependent. However, our results and the results of others combined do not explain how disease severity in patients correlates with specific mutations of TRPV4 channel proteins.

Conclusion

Although recent studies have made great strides in piecing together the puzzle of TRPV4 channelopathies and skeletal dysplasias, many aspects of the disease process remain to be elucidated. It is likely

that different cellular signaling mechanisms may cause different subsets of disease pathology, e.g., some channelopathy mutations are likely de novo targets for phosphorylation. Modeling work could possibly help to clarify the effects of different TRPV4 mutations on the structure of the protein. The TRPV4 amino-terminus has been elucidated structurally, and the TRPV1 cryo-electron microscopy structure is now available. A number of structurally related K-channels have been resolved crystallographically as well, so that, now, structures of TRPV4 channelopathy mutations can be illustrated by homology modeling, yielding a novel angle of insight.

Ultimately, the goal is to first understand how these mutations cause disease, then to develop effective interventions for these skeletal dysplasia patients. In this respect, we established the promising finding that blocking mutant TRPV4 channels with a small molecule TRPV4 inhibitor prevented excessive FST production.[22] Given that TRPV4 inhibitors have been used therapeutically in other settings,[3,31] it is possible that appropriately timed delivery of a TRPV4 inhibitor may restore normal endochondral ossification toward physiological skeletal development. In addition to chemical agonists, inhibiting TRPV4 via gene therapy approaches, such as dominant-negative TRPV4 or transitory RNAi expression,[32] may provide another therapeutic avenue.

In conclusion, 6 years after the initial description of a TRPV4 channelopathy mutation, our study,[22] combined with others,[23,24] has yielded new insights into the mechanisms by which TRPV4 mutations cause skeletal dysplasias. We have shown that TRPV4 channelopathies cause skeletal dysplasias by inducing a Ca^{2+}-dependent upregulation of FST in chondrocytes, which inhibits BMP signaling in the developing skeleton, thus preventing chondrocytes from undergoing normal hypertrophy, inhibiting endochondral ossification, and ultimately resulting in skeletal dysplasia.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Leddy, H. A., McNulty, A. L., Lee, S. H., Rothfusz, N. E., Gloss, B., Kirby, M. L., Hutson, M. R., Cohn, D. H., Guilak, F., and Liedtke, W. Follistatin in chondrocytes: the link between TRPV4 channelopathies and skeletal malformations. FASEB Journal Published online before print February 27, 2014, doi: 10.1096/fj.13-245936 fj.13-245936

Funding

This work was supported in part by the US. National Institutes of Health grants AR48182 (FG), AR48852 (FG), AG15768 (FG), AR50245 (FG), AG46927 (FG), and DE018549 (WL).

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