WNK2 Regulates the Response to Hyperosmotic Stress in Chondrocytes

Shivakumar R. Veerabhadraiah[1], D.J. Matheson[1], M. Honeggar[1], C. Aslor[1], N.H Kazmers[1], D.J. Grunwald[2], **Mick J. Jurynec**[1,2]

[1]Department of Orthopaedics and [2]Human Genetics, University of Utah, Salt Lake City, UT

jurynec lab.org

Introduction

Osteoarthritis (OA) is a debilitating disease affecting millions worldwide. Despite its prevalence, there are currently no effective disease-modifying therapies. The main obstacle in developing such therapies is a poor understanding of the mechanisms driving the development of OA. Our goal is to discover genes and molecular pathways in humans that are vulnerability points in the development of OA and generate mouse models with human disease alleles.

The osmolarity of the synovial joint varies considerably in response to everyday use and joint damage. How chondrocytes of the synovial joint sense and respond to changes in osmolarity to maintain homeostasis is undetermined. We hypothesized pathways that maintain osmotic responses would be critical in OA pathogenesis. Our initial goal was to identify whether genetic variants potentially affecting the response to osmotic stress were associated with susceptibility to OA.

We study many unrelated families with clear-cut inherited forms of OA to identify susceptibility alleles that have strong determinate effects. We have analyzed the exomes of 151 families with multiple forms of OA and identified independent rare coding variants in the With No Lysine (K) Kinase 2 (WNK2) gene.

Here we test the role of WNK2 in chondrocytes using both gain- and loss-of function analyses. Our data indicate altered WNK2 signaling confers susceptibility to OA. We hypothesize the synergistic effects of hyperosmotic stress and high WNK2 activity promote development of OA and identify WNK2 signaling as a major risk factor for OA susceptibility.

WNK2 is a kinase that responds to hyperosmotic stress

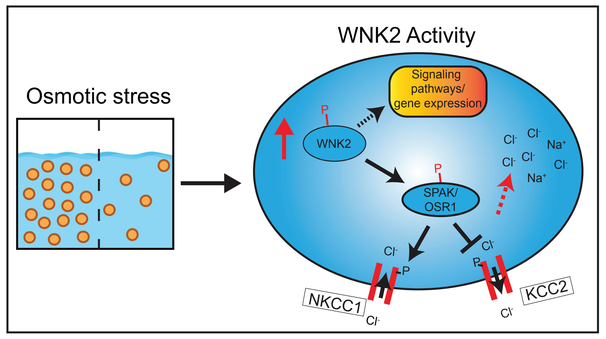


Figure 1: The With-No-Lysine (K) 2 (WNK2) protein kinase is an intracellular sensor that respond to hyperosmotic stress by regulating ion channel activity and signaling pathways. In response to acute hyperosmotic stress, WNK2 phosphorylates and activates the kinases SPAK and OSR1, which in turn phosphorylate and activate sodium and potassium chloride co-transporters to modulate osmoregulation. Though none of the WNKs have been associated previously with OA, ion channels targeted by WNKs are expressed in chondrocytes and upregulated in human OA tissue.

Mutations in *WNK2* are associated with familial osteoarthritis (OA)

Analyzed ~151 families

Gene Discovery

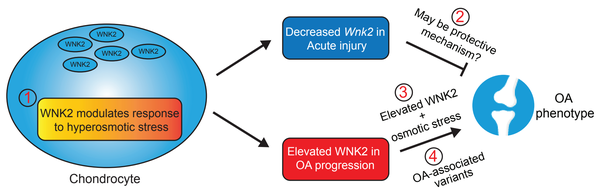
Families with dominant osteoarthritis (OA)

Identify common genes/pathways

With-No-Lysine (K) 2 (WNK2) variants identified in OA families

OA Phenotype (Family number)	Variant	Minor Allele Frequency	Protein Domain Affected by Variant
1st MTP Joint OA (MTP24) and Erosive Hand OA (ERO32)	c.C2272A:p.H758N	0.0048	Q-rich domain
Erosive Hand OA (ERO549024)	c.G6161A:p.R2054Q	0.0000073	Q-rich domain
Erosive Hand OA (ERO20)	c.C3013T:p.L1005F	0.000013	Q-rich domain

All affected individuals have OA in miltiple joints



We hypothesize:

1. That WNK2 signaling is central in the chondrocyte response to hyperosmotic stress.

2. That alteration of WNK2 activity may be critical for OA pathogenesis.

Results

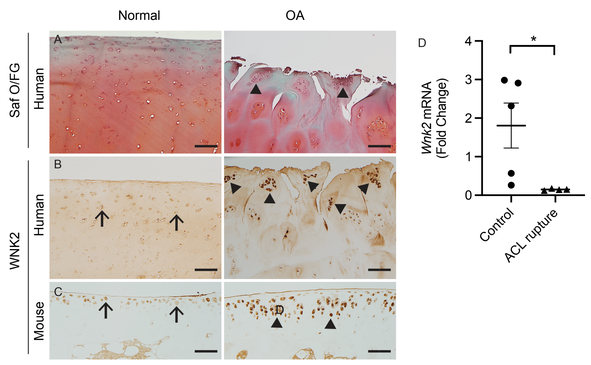


Figure 2: WNK2 expression is elevated in chondrocytes present in injured mouse joints and human osteoarthritic tissue. (A) Safranin O/Fast Green staining of healthy (Normal) and osteoarthritic (OA) human cartilage from the humeral head. **(B&C)** Immunohistochemical staining reveals low WNK2 expression in healthy human chondrocytes and uninjured mouse tibia, contrasting with high expression in hypertrophic chondrocytes in damaged human OA cartilage and 8 weeks post-ACL rupture in the mouse tibia. Arrows indicate normal chondrocytes, and arrowheads mark hypertrophic chondrocytes. Scale bar = 100µm. **(D)** *Wnk2* is acutely downregulated after ACL rupture, as shown by qPCR analysis in mouse knee joints 5 days post-rupture. Statistical significance (*P ≤ 0.05); two-tailed unpaired t-test, n=4.

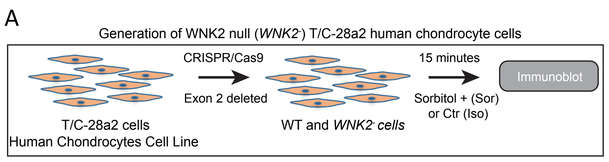


Figure 3: WNK2 mediated the response to hyperosmotic stress in chondrocytes. (A) I 2 (2) **(B)** *WNK2*- chondrocytes show no response to acute hyperosmotic stress. Immunoblot analysis of total SPAK and phosphorylated SPAK (pSPAK) in WT and *WNK2*- chondrocytes under hyperosmotic stress. Ratio indicates pSPAK:total SPAK levels. Iso – isotonic conditions, Sor – sorbitol concentration in cell culture media.

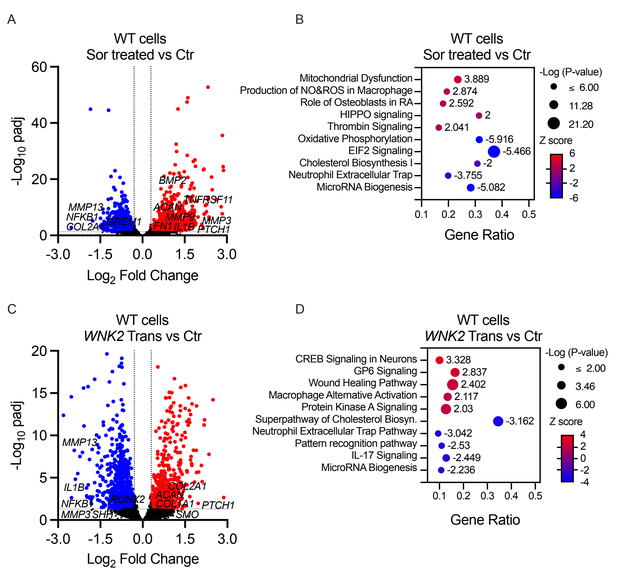


Figure 4. Chronic hyperosmotic stress or *WNK2* overexpression induces a remodeling response in chondrocytes. RNA-seq analysis of chondrocytes treated for 7 days with 100mM sorbitol (Sor) or transfected (*WNK2* Trans) chondrocytes. Volcano plots **(A, C)** show significantly upregulated (red) or downregulated (blue) genes in WT chondrocytes compared to control (Ctr). Bubble plots **(B, D)** depict top KEGG pathways from differentially expressed genes. x-axis: gene ratio; y-axis: KEGG pathways. Each bubble represents a specific pathway, with the color of the bubble indicating the positive (increased pathway gene expression, red) or negative (decrease pathway gene expression, blue) Z score. Darker colors signify higher significance. Bubble size corresponds to -Log (P-value) indicating the level of significant enrichment..

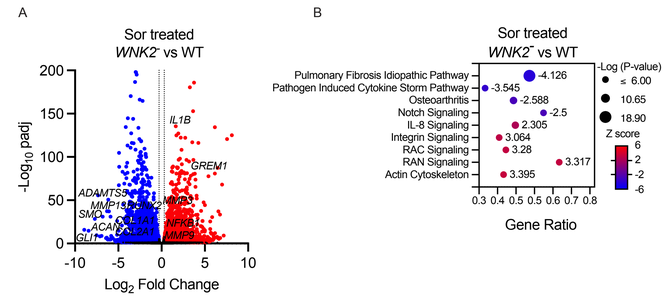


Figure 5. WNK2 is necessary for OA-associated gene expression in chondrocytes exposed to chronic hyperosmotic stress. Volcano plot **(A)** show significantly upregulated (red) or downregulated (blue) genes in *WNK2*- chondrocytes exposed to chronic hyperosmotic stress. Bubble plots indicate that many pathways associated with OA are downregulated in *WNK2*- chondrocytes.

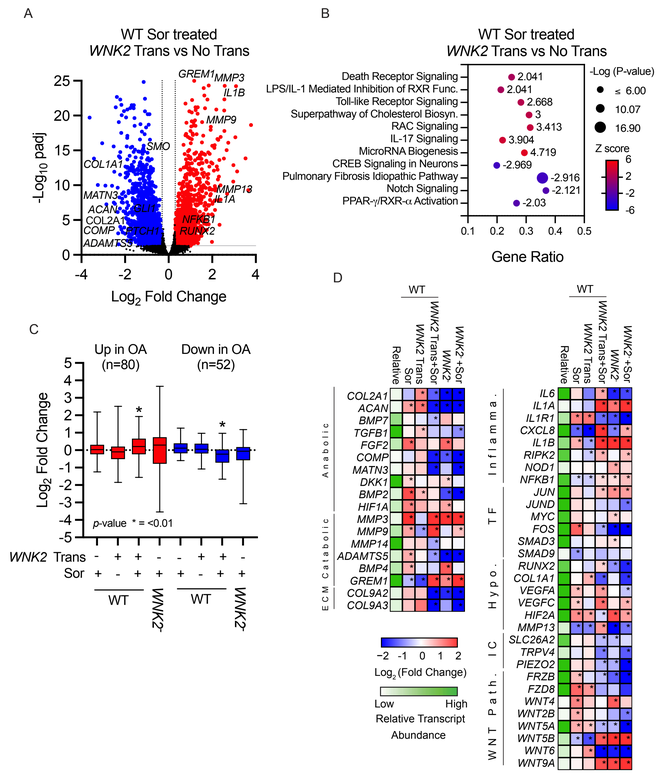


Figure 6. WNK2 acts synergistically with chronic hyperosmotic stress to induce expression of OA-associated genes and pathways. Comparative RNA-seq analysis of WT and *WNK2* transfected chondrocytes exposed to chronic hyperosmotic stress. (**A**) Volcano plots show genes significantly upregulated (red) or downregulated (blue) in WT chondrocytes transfected with WNK2 and treated with 100mM sorbitol (WNK2 Trans) compared to control chondrocytes treated with 100mM sorbitol (No Trans) for 7 days. (**B**) Bubble plots illustrate top KEGG pathways from differentially expressed genes in (**A**). (**C**) Common set of differential expressed genes (DEGs) identified from 5 human OA gene expression studies, with 82 up-regulated and 52 down-regulated DEGs shared between at least 3 studies. Genes were ranked by fold change and compared to a ranked list of fold changes for genes outside of the OA set (* = Mann-Whitney P-value ≤ 0.01). (**D**) Expression of selected OA-associated genes in WT and *WNK2*- transfected (Trans) or sorbitol (Sor) treated cells. Blue tiles indicate repression, red tiles indicate induction of gene expression. ECM – Extra cellular matrix, Inflamma. – Inflammation, TF – transcription factors, Hypo – genes associated with hypertrophy, and IC – ion channels.

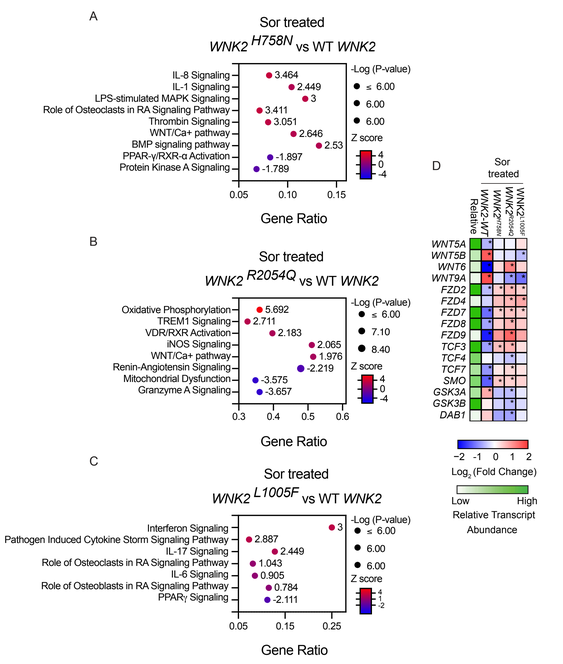


Figure 7. The OA-associated WNK2 coding variants augment the synergistic response of WNK2 and hyperosmotic stress to promote expression of OA-associated genes and pathways. Comparative analysis of RNA-seq performed on WT chondrocytes overexpressing WT *WNK2*, *WNK2*^{H758N}, *WNK2*^{R2054Q}, or *WNK2*^{L1005F} and exposed to hyperosmotic stress. **(A-C)** Bubble plots illustrate the top KEGG pathways identified from the differentially expressed genes. **(D)** Expression of selected *Wnt* pathway genes altered by *WNK2* overexpression and hyperosmotic stress. The WT *WNK2* overexpressing chondrocytes were compared with WT chondrocytes and the *WNK2* variant overexpressing chondrocytes were compared with WT chondrocytes overexpressing WT *WNK2*.

Model

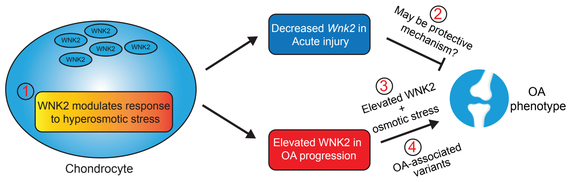


Figure 8. Model of WNK2 signaling in chondrocytes during homeostasis and OA. Based on our data, we propose that WNK2 singling functions in normal chondrocytes to sense and respond to hyperosmotic stress. During acute injury, WNK2 is downregulated, which may be be a mechanism to protect chondrocytes from injury and reduce expression of OA-associated genes and pathways.

In contrast, risk factors (aging, environment, genetics) may lead to the upregulation of WNK2 in chondrocytes prior to overt development of OA. This upregulation, in combination with the normally hyperosmotic environment of the joint, induces expression of pro-OA genes and pathways. This alteration in gene expression may lead to the disruption of joint homeostasis and development of OA.

Conclusions

- We identified *WNK2* mutations that are associated with dominant forms of familial OA.
- WNK2 is acutely downregulated in early stages of injury-induced OA but upregulated in advanced stages.
- Chondrocytes initiate a remodeling response to hyperosmotic stress or *WNK2* overexpression by regulating both anabolic and catabolic genes and pathways.
- Loss of WNK2 function in chondrocytes inhibits OA-associated gene expression.
- *WNK2* and hyperosmotic stress act synergistically to induce a transcriptional response associated with OA and the OA-associated variants augment this response.
- Currently studying the role of WNK2 *in vivo*: we have generated a *Wnk2* null mouse and a mouse harboring one of the human OA-associated alleles (*Wnk2*^{R2054Q})
- **In sum,** we propose that WNK2 is a major hyperosmotic sensor in chondrocytes that maintains joint homeostasis. WNK2 and chronic hyperosmotic stress act synergistically to promote OA-associated gene expression. Finally, we propose that altered WNK2 activity confers susceptibility to OA.

This work was supported by funding from the NIH, ANRF, the Utah Genome Project, and the Skaggs Foundation for Research.