

WNK2 regulates the response to hyperosmotic stress in chondrocytes

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INTRODUCTION: OA is a common joint disease characterized by abnormal remodeling of joint tissue, affecting 32.5 million adults in the US without a cure or effective treatment. Emerging evidences suggests that changes in synovial joint osmolarity plays a crucial role in the development and progression of OA, but the molecular mechanisms that sense and respond to osmotic stress are unknown. Our lab takes a unique approach to identify genes and pathways that contribute to OA susceptibility in humans. 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. WNK2 variants were associated with hand OA. WNK2 is a serine/threonine-protein kinase that senses and responds to osmotic stress. We found that WNK2 is differentially expressed during early and late stages of OA in human tissue and in a mouse model of OA. This study aimed to elucidate the precise roles of WNK2 in OA pathogenesis. We examine the genes and signaling pathways regulated by WNK2 in chondrocytes. Here we describe the identification of the WNK2 signaling pathway as a major risk factor for OA susceptibility.

METHODS: We used the Utah Population Database, to identify 151 independent families with dominant inheritance patterns of OA. Whole exome sequence (WES) analysis was performed on informative family members. To determine WNK2 expression in normal and OA tissues of human and mouse, human humeral heads were collected during total shoulder arthroplasties and DMM was performed on C57BL/6J mice to induce OA. WNK2 null T/C-28a2 cells (WNK2⁻) were created using CRISPR/Cas9 genome editing and validated using Sanger sequencing. To test if WNK2 regulates the hyperosmotic stress response in chondrocytes, we examined the dose-response of phospho-SPAK (pSPAK) in wild-type (WT) and WNK2⁻ chondrocytes. Cells were treated with isotonic media or media containing 50, 200, and 500 mM sorbitol for 15 minutes and protein extracts were collected for immunoblot analyses. To determine the transcriptional response of osmotic stress and/or *WNK2* overexpression in chondrocytes, WT and WNK2⁻ chondrocytes were transfected with a *WNK2* plasmid and treated with sorbitol to modulate osmolarity and incubated for 7 days. RNA was collected 7 days after treatment and used for RNA-seq analyses. Data analysis included quality control, alignment, fold-change calculation, and differential expression analysis based on an adjusted p-value of 0.05. The differentially expressed genes (DEGs) in each dataset was used to perform Ingenuity Pathway Analysis (IPA) to investigate the dysregulated pathways associated *WNK2* overexpression and/or osmotic stress.

RESULTS: In normal human humeral head cartilage, WNK2 is present at low levels in chondrocytes (**Figure 1 top-left**). In contrast, it is strongly expressed in hypertrophic chondrocytes in damaged human osteoarthritic tissue (humeral head) harvested at the time of total shoulder arthroplasty (**Figure 1 top-right**). Consistent with these findings, WNK2 is expressed in the superficial layer of articular chondrocytes in the uninjured mouse knee joint (**Figure 1 bottom-left**), and it is highly expressed in the deep layer of hypertrophic chondrocytes 8 weeks after induction of OA (**Figure 1 bottom-right**). These data indicate that WNK2 expression is upregulated in diseased tissue and maybe contribute to OA onset or progression. WT chondrocytes have a basal level of pSPAK and respond to osmotic stress by increasing levels of pSPAK relative to total SPAK levels (pSPAK:SPAK ratio). In the presence of isotonic or 50 mM sorbitol in media WNK2⁻ chondrocytes have similar levels of pSPAK:SPAK as WT chondrocytes. However, when challenged with 200 or 500 mM sorbitol, WNK2⁻ chondrocytes have almost no detectable pSPAK (**Figure 2**), indicating the WNK2 is the major osmotic sensor in chondrocytes. To determine the genes and pathways regulated by WNK2 overexpression in combination with sorbitol treatment, we analyzed the transcriptional response in chondrocytes using RNA-seq. We identified many genes that are associated with OA pathogenesis and a downregulation of anabolic genes (**Figure 3**). The top pathways upregulated by WNK2 overexpression and sorbitol treatment are microRNA biogenesis, IL-17 signaling, and RAC signaling, while CREB signaling, pulmonary fibrosis idiopathic signaling, and Notch signaling pathways are downregulated. This response is dependent on WNK2 function as these pathways are not altered in WNK2⁻ chondrocytes with or with sorbitol treatment. In sum our data indicate that WNK2 is the major sensor of osmotic stress in chondrocytes and when overexpressed with osmotic stress, WNK2 regulates many gene associated with OA pathogenesis.

DISCUSSION: We found that WNK2 overexpression and sorbitol treatment significantly altered transcriptional changes resembling those associated with the OA phenotype. A dysregulation of genes associated with cartilage homeostasis, inflammation, and other cellular processes may contribute to OA pathogenesis through WNK2 and osmotic stress. We have generated a *Wnk2* null mouse and a mouse harboring a human OA-associated WNK2 allele and we are currently analyzing joint homeostasis in these mice.

SIGNIFICANCE/CLINICAL RELEVANCE: Our data provide strong support that WNK2 is critical for the chondrocyte response to hyperosmotic stress, which will advance our understanding of OA initiation and progression. This study could provide a scientific basis for establishing new targets for the prevention and treatment of OA.

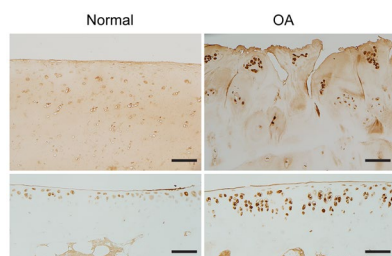


Figure 1 – WNK2 expression is elevated in injured mouse joints and in human osteoarthritic tissue. Immunohistochemistry indicating WNK2 expression in human cartilage tissue from normal and OA patients (**Top**) and uninjured (Normal; left tibia) and injured mouse (OA; right tibia 8 weeks post ACL injury) (**Bottom**). Scale bar = 100 µm.

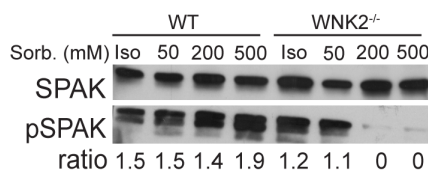


Figure 2 – WNK2 regulates the response to hyperosmotic stress in chondrocytes. Immunoblot depicting total SPAK and pSPAK levels in WT and WNK2 null (Wnk2⁻) T/C-28a2 chondrocytes in response to hyperosmotic stress. Ratio indicates the levels of pSPAK: SPAK.

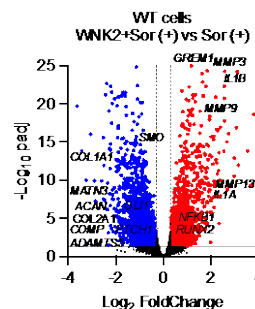


Figure 3 – WNK2 overexpression combined with sorbitol treatment induces changes similar to OA. The volcano plot indicates genes significantly upregulated (red) or downregulated (blue). WNK2 overexpression combined with sorbitol treatment in WT chondrocytes compared sorbitol treatment in WT chondrocytes.