Human genetics to drugs: Using familial osteoarthritis to identify novel pathways for drug discovery and validation

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INTRODUCTION: Osteoarthritis (OA) is a common joint disease characterized by abnormal remodeling of joint tissue. Despite our understanding of the molecular and cellular processes that contribute to OA susceptibility, we lack bona fide genetic targets for drug development. To discover promising drug targets, our lab has taken a unique approach to identify genes and pathways that contribute to OA susceptibility in humans. We study many unrelated families with dominant 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 and foot OA. WNK2 is a serine/threonine-protein kinase that senses and responds to osmotic stress. Cells of the synovial joint must sense and respond to changes in osmolarity to maintain joint homeostasis. Emerging evidence 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 published data demonstrated that WNK2 mediates the response of chondrocytes to hyperosmotic stress, and the combination of elevated WNK2 expression and hyperosmotic stress promotes an OA-associated transcriptional response. Furthermore, the expression of the WNK2 variants in the absence of hyperosmotic stress is sufficient to promote expression of pathways associated with OA, a response that is further amplified under conditions of hyperosmotic stress. To test the role of WNK2 *in vivo*, we generated a mouse model harboring the human $Wnk2^{R2054Q}$ allele and a *Wnk2* null ($Wnk2^{-/-}$) mouse. Our *in vivo* work indicated that $Wnk2^{R2054Q}$ accelerates OA development in an injury model through the enhancement of proinflammatory signaling pathways. The goal of our work is to discover and validate novel inhibitors of WNK2 and determine if WNK2 interacts with other pathways associated with familial OA.

METHODS: We used the Utah Population Database, to identify 151 independent families with dominant inheritance patterns of OA. Whole exome sequence analysis was performed on informative family members. Given that there are no specific WNK2 inhibitors, we employed a comprehensive computational approach to identify novel chemical inhibitors specific to WNK2. We utilized structure-based virtual screening targeting the WNK2 protein kinase domain (PKD) binding pockets using Mcule and Cheminfo. Further these selected inhibitors were docked using Autodock suite and Molecular Operating Environment (MOE) to identify the most promising candidates. We examined the effect of candidate inhibitors on WNK2 kinase activity and determined specificity. We tested the effect of WNK2 inhibition on a chondrocyte cell line and on primary human chondrocytes, including cell viability and the gene expression response to IL1B stimulation. To determine if WNK2 interacts with other pathways associated with familial OA, we generated mice harboring a combination of $Wnk2^{R2054Q}$;$Ripk2^{104Asp}$ and $Wnk2^{R2054Q}$;$Piezo1^{R1398}$. We examined the transcriptional response to injury induced OA in these mice to determine if the double mutants had an increased susceptibility to OA.

RESULTS: Our *in silico* screening identified 53 candidate compounds with binding energies ranging from -4 to -8 kcal/mol. 15 inhibitors were selected for experimental validation to assess their ability to inhibit WNK2 kinase activity. M04 emerged as a potent and specific inhibitor of WNK2. The structural analysis of M04 showed that it binds WNK2 specificity through ionic interactions with the unique residue Glu249. Hydrophobic interactions from its fluorinated rings and polar contacts involving Lys244 and Lys251 also contributed to this stability. M04 effectively reduced pSPAK (a direct target of WNK2) levels in a dose-dependent manner (Figure 1). It exhibited no cytotoxic effects on human chondrocyte T/C-28a2 cells, even at high concentrations of 100 uM for 24 hrs, highlighting its safety profile. Furthermore, protein kinase assays confirmed the specificity of M04 for WNK2 over other WNK family kinases (Figure 2). Treatment of T/C-28a2 and primary human chondrocytes with MO4 reduced IL1B-induced proinflammatory gene expression. Our previous work has identified mutations in *RIPK2* and *PIEZO1* associated with familial OA. Our molecular analysis of the $Wnk2^{R2054Q}$, $Ripk2^{104Asp}$, and $Piezo1^{R1398}$ mice indicated that all three variants regulated a common pathway – inflammation. To test if these genes interact to regulate OA susceptibility, we generated $Wnk2^{R2054Q}$;$Ripk2^{104Asp}$ and $Wnk2^{R2054Q}$;$Piezo1^{R1398}$ mice. The mice are phenotypically normal. We tested if the double mutant mice have susceptibility to injury induced OA and increased proinflammatory gene expression. DMM surgery was performed and RNA from whole knee joints was used for RNAseq analysis 14 days post injury.

DISCUSSION: We found that increased WNK2 activity is associated with OA susceptibility through upregulation of proinflammatory gene expression. As there are no specific WNK2 inhibitors, we used *in silico* approaches to identify and characterize a novel inhibitor of WNK2. We are currently testing if this inhibitor reduces OA severity in a mouse model. Future studies will examine the genetic interaction of WNK2 with other OA susceptibility pathways to define shared biological pathways for future drug discovery and development.

SIGNIFICANCE/CLINICAL RELEVANCE: Our data provides strong support that WNK2 has a central role in maintaining joint homeostasis. We have identified novel chemical inhibitors of WNK2, which may be a new avenue for OA therapeutics.

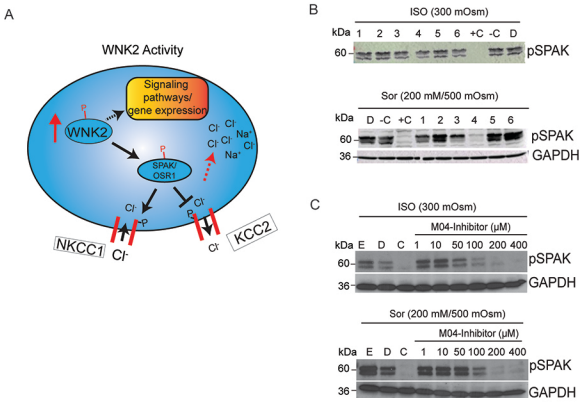

Fig. 1 WNK2 activity and M01 to M06 inhibitions on WNKs using pSPAK level under osmotic stress conditions. (A) Graphical representation of WNK2 activity. WNK2 phosphorylates SPAK and OSR1 in response to osmotic stress. (B) Activity of WNK2 inhibitors.

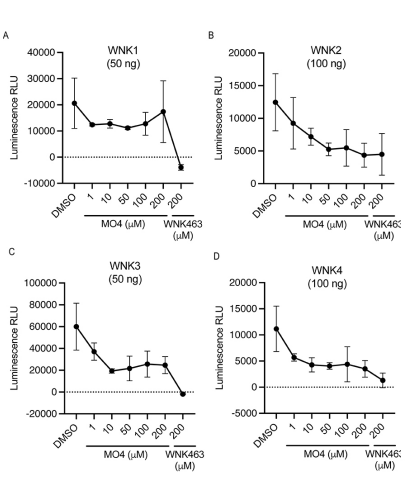

Fig. 2 Specificity of MO4. Kinase activity of WNK1 (A), WNK2 (B), WNK3 (C), and WNK4 (D) was measured using the ADP-Glo™ Kinase Assay. Luminescence (relative light units, RLU) was measured after subtraction of background signal (black). Kinase reactions were performed in the presence of DMSO (vehicle control), increasing concentrations of the inhibitor M04 in (1, 10, 50, 100, and 200 μM), or the WNK-specific inhibitor WNK463 (200 μM) (positive control). Dose-dependent decreases in luminescence indicate inhibition of kinase activity by M04. Data represent the mean ± SD from [n=4] independent experiments.