The Spatiotemporal Requirement of Hyperactive RIPK2 Activity on Osteoarthritis Development

#455

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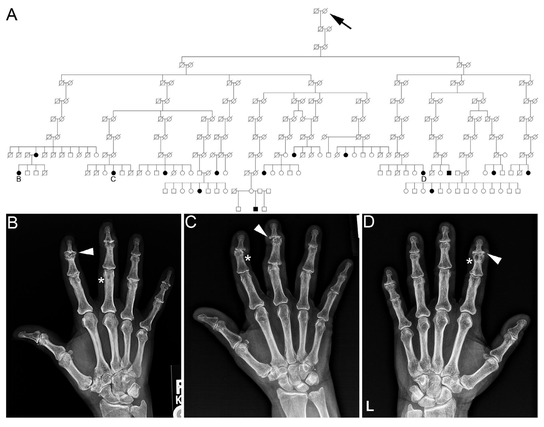
Background

The main obstacle to the development of disease-modifying therapies for OA is poor understanding of the disease process and lack of appropriate genetic models. How joint homeostasis can be disrupted leading to OA is unknown. We previously identified a hyperactive allele of *RIPK2* (*RIPK2104Asp*) associated with familial forms of OA. We generated a mouse harboring this allele and demonstrated that the human OA-associated *Ripk2104Asp* allele acts dominantly in mice to elevate inflammatory signaling in the knee joint. This elevated inflammatory signaling causes increased susceptibility to both injury-induced and age-associated OA. However, these data do not give us insight into the cellular basis of the regulatory network that senses damage and responds to it. Our goal is to address a fundamental question in the OA field: is a hyperactive inflammatory response in structural tissues and/or immune cells sufficient for initiating OA. To determine if expression of *Ripk2104Asp* in specific cell types is capable of increasing susceptibility to OA, we generated an allele of *Ripk2104Asp* that can be conditionally activated. We will .We determine if *Ripk2104Asp* activity in specific cell types is sufficient to alter homeostasis of the knee joint by performing histological and immunohistochemical analyses and gene expression analyses to determine if tissue specific activation of *Ripk2104Asp* disrupts homeostasis of the joint as observed in *Ripk2104Asp* mice.

Identification of families with OA from the Utah Population Database



Mutations in the NOD/RIPK2 are associated with familial OA

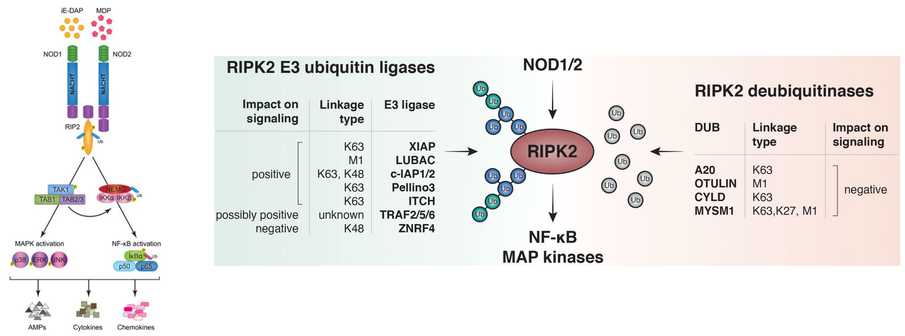
NOD/RIPK2 pathway

Table 1. *NOD/RIPK2* Pathway Variants Identified in Independent Osteoarthritis Families

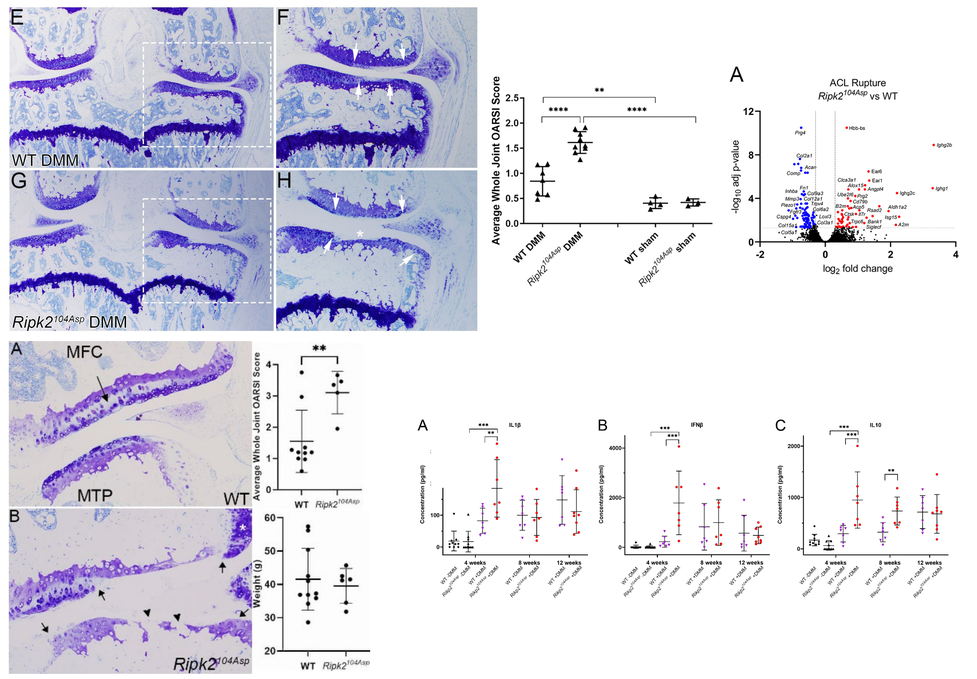
Gene	OA Phenotype (Family)	Variant	Minor Allele Frequency	Protein Domain Affected by Variant
NOD1	Finger Interphalangeal Joint OA (FIJ744)	c.G2114A:p.R705Q	0.0008	Leucine Rich Repeat Domain
NOD2	1st MTP Joint OA (UUHR2)	c.C2546T:p.A849V	0.00007	Leucine Rich Repeat Domain
NOD2	Finger Interphalangeal Joint OA (FIJ7)	c.G247A:p.A83T	0.00008	Caspase Activation and Recruitment Domain
IKBKB	Glenohumeral OA (SA735)	c.G1663A:p.G555R	0.00008	Scaffold Dimerization Domain
CARD9	Finger Interphalangeal Joint OA (FIJ9)	c.G722A:p.R241Q	0.00005	Structural Maintenance of Chromosomes
CHUK	1st MTP Joint OA (MTP25)	c.A376T:p.S126C	0.0008	Kinase Domain
*RIPK2**	1st MTP Joint OA (UUHR1)	c.A310G:pN104D	0.0004	Kinase Domain

* - Previously described in Jurynec, 2018.

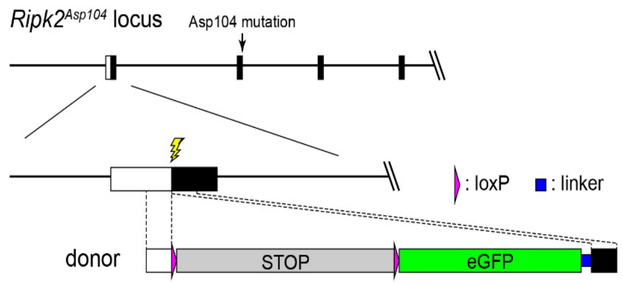
Biological function of the NOD/RIPK2 pathway



The Ripk2^{104Asp} allele acts dominantly and is sufficient to confer increased susceptibility to OA

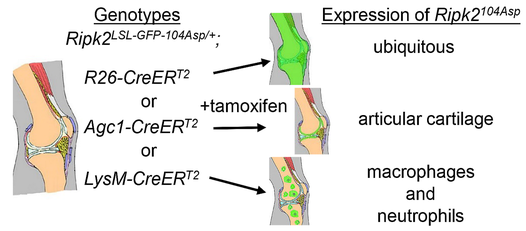


The conditional Rikp2^{Asp104} disease allele

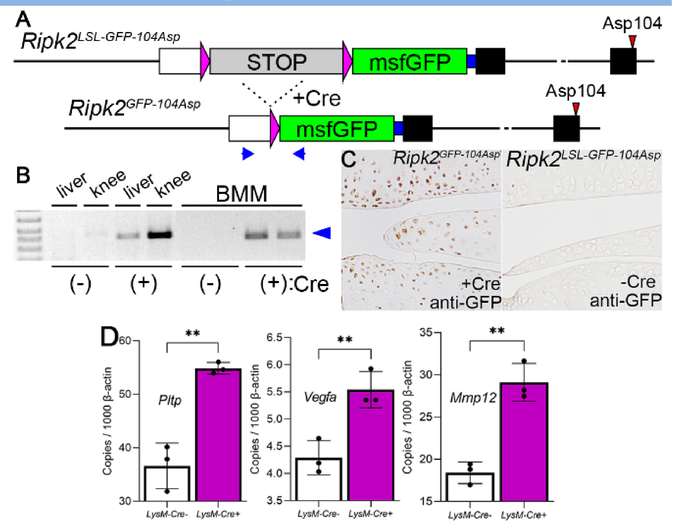


We used CRISPR/Cas9 to create a double strand break (lightning bolt) and insert a *loxP*-stop-*loxP*-eGFP cassette immediately upstream of the *Ripk2*^{104Asp} coding sequence. The Asp104 disease allele remains inactive until Cre-mediated recombination excises the stop cassette, resulting in expression of a GFP-tagged Ripk2^{Asp104} protein. Expression of GFP allows us to track the cells and tissues that express the *Asp104* disease allele.

Schematic overview of experimental design to express the Ripk2^{104Asp} disease allele in specific cells and tissues



The Ripk2^{104Asp} conditional allele is functional.



(**A**) The *Ripk2*^{104Asp} allele is activated with Cre. (**B**) PCR on cDNA generated from the liver, knee, and BMM from tamoxifen/control injected *R26-CreER*^{T2}*;Ripk2*$^{+/LSL-GFP-104Asp}$ mice. (**C**) GFP IHC to detect GFP-Ripk2 in the cartilage and meniscus of knees from tamoxifen/control injected *R26-CreER*^{T2}*;Ripk2*$^{+/LSL-GFP-104Asp}$ mice. GFP-Ripk2 is **not** expressed in Cre- knees (**D**) qPCR on BMM cDNA generated from tamoxifen/control injected *LysM-CreER*^{T2}*;Ripk2*$^{+/LSL-GFP-104Asp}$ mice. Activation of GFP-Ripk2 results in increased expression of *Pltp*, *Vegfa*, and *Mmp12* relative to BMM expressing WT Ripk2. $p < 0.01$, Students t-test.

Conclusions

Our studies demonstrate that our conditional *Ripk2*^{104Asp} is functionally equivalent to the endogenously expressed *Ripk2*^{104Asp}. Further analysis of *Ripk2*^{104Asp} activity in different cell/tissue type will define the requirement of hyperactive RIPK2 signaling in OA development and inform rational therapeutic design.

Developing new cell/tissue specific OA animal models with human susceptibility alleles is useful for understanding mechanism of disease, identifying and developing biomarkers for early detection of OA, and therapeutic development.

Acknowledgements

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