Lacritin from Tears Restores Chondrocyte Homeostasis and Protects Against Osteoarthritis Progression

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Disclosures: We have nothing to disclose.

INTRODUCTION: Osteoarthritis (OA) is a progressive chronic conditions and the most common form of disabling arthritis, affecting more than 32.5 million adults in the US. Despite the public health burden, there are no disease-modifying treatment is currently available. Lacritin, a glycoprotein originally identified in tears and saliva and later found in plasma, cerebrospinal fluid, and urine, has been implicated in maintaining tissue homeostasis across multiple organ systems. Its receptor, syndecan-1 (SDC1), is expressed in both human and mouse chondrocytes, suggesting a potential role for the lacritin–SDC1 axis in cartilage biology. N-104 is a C-terminal fragment of lacritin that contains a positively charged amphipathic α-helix, enabling it to bind glycosaminoglycans in cartilage and exert chondroprotective effects (**Fig. 1a**). In this study, we hypothesize that N-104 exerts chondroprotective effects by engaging the lacritin–SDC1 axis to restore chondrocyte homeostasis, inhibit apoptosis, and preserve joint integrity, thereby preventing OA progression and alleviating OA-associated pain.

METHODS: Primary mouse joint explants and chondrocytes were dissected from postnatal day 5 C57BL/6 mice and cultured at passages 0–1. Chondrocytes and joint explants were treated with N-104 peptide (1 μM) for 3 days, followed by assessment of glycosaminoglycan (GAG) release, mRNA expression of aggrecan and collagen II in cells and proteoglycan retention via Safranin-O staining in explant cultures. To evaluate in vivo efficacy, osteoarthritis was induced in 12-week-old male C57BL/6 mice using the destabilization of the medial meniscus (DMM) surgical model. Mice received intra-articular injections of N-104 (5 μM in 10 μL) or saline twice per week for up to 8 weeks. Pain sensitivity was assessed longitudinally by von Frey assay. At study endpoints, knee joints were collected for histological evaluation with Alcian Blue/Picrosirius Red staining and OARSI scoring, alongside tartrate-resistant acid phosphatase (TRAP) and TUNEL staining to assess osteoclast activity and chondrocyte apoptosis, respectively. Further analyses, including detailed joint histology and microCT assessment of subchondral bone remodeling, are ongoing.

RESULTS: Immunostaining confirmed the presence of lacritin's receptor, syndecan-1 (SDC-1), in primary mouse chondrocytes (**Fig. 1b**). At 1 μM, N-104 significantly reduced GAG release into culture medium (**Fig. 2a**), upregulated mRNA expression of aggrecan and collagen II in primary chondrocytes (**Fig. 2b**), and reduced the loss of proteoglycan in cultured knee joint explants after 3 days (**Fig. 2c**). In vivo, intra-articular injection of N-104 twice weekly started on the day of DMM surgery (**Fig. 3a**). Von Frey assays revealed that N-104 attenuated the DMM surgery-induced decline in paw withdrawal threshold (PWT) of ipsilateral joints across weeks 2–8 (**Fig. 3b**). Histological evaluation further demonstrated that N-104 reduced osteoarthritic changes, as decreased chondrocyte loss, and enhanced retention of matrix-producing chondrocytes in the medial tibial plateau (**Fig. 3c**). Results from other outcome measures are ongoing in our laboratory. Together, these findings suggest that tear peptide N-104 protects knee joints from posttraumatic osteoarthritis.

DISCUSSION: N-104 engages the lacritin–SDC1 axis to restore chondrocyte homeostasis, reduce apoptosis, and protect against cartilage degeneration and pain in a posttraumatic osteoarthritis mouse model. We have incorporated SDC1 knockout (SDC1KO) mice to confirm the receptor-specific mechanism of N-104 activity, which are currently ongoing in our laboratory.

SIGNIFICANCE/CLINICAL RELEVANCE: These findings highlight N-104 as a promising disease-modifying therapeutic candidate for osteoarthritis, with potential to address both structural damage and pain beyond current palliative treatments.

ACKNOWLEDGEMENTS: We are grateful for financial support from NIH NIAMSR21AR072334.

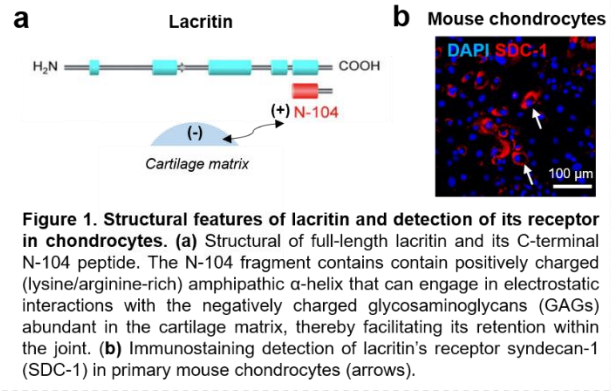


Figure 1. Structural features of lacritin and detection of its receptor in chondrocytes. (a) Structural of full-length lacritin and its C-terminal N-104 peptide. The N-104 fragment contains contain positively charged (lysine/arginine-rich) amphipathic α-helix that can engage in electrostatic interactions with the negatively charged glycosaminoglycans (GAGs) abundant in the cartilage matrix, thereby facilitating its retention within the joint. **(b)** Immunostaining detection of lacritin's receptor syndecan-1 (SDC-1) in primary mouse chondrocytes (arrows).

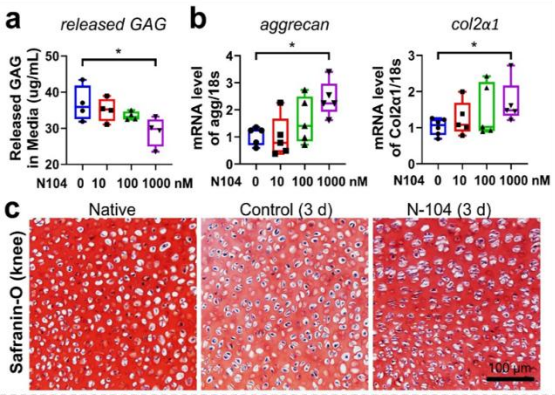
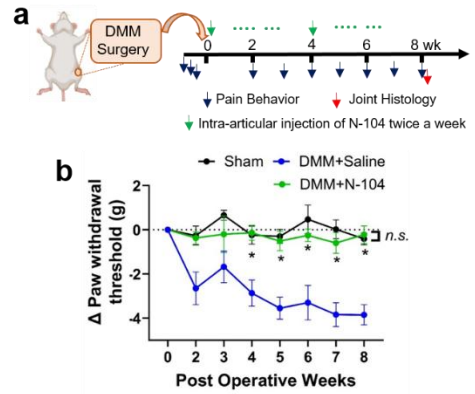


Figure 2. N-104 promotes anabolic metabolism in mouse primary chondrocytes and knee explants after 3 days culture. At 1 μM, N-104 reduced released GAG content in chondrocytes media **(a),** increased mRNA levels of aggrecan and collagen II in chondrocytes **(b),** and decreased loss of proteoglycan in knee joints explant culture after 3 days **(c)**. * $p<0.05$ by t-test, n=4-5 biological replicates per group.



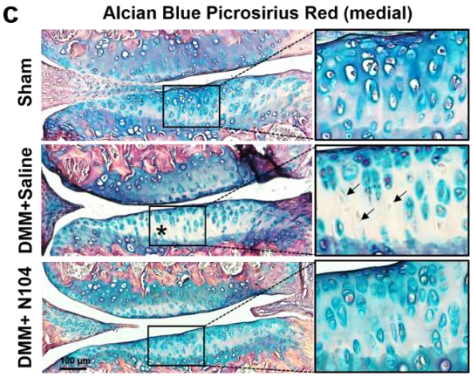


Figure 3. N-104 alleviates pain sensitivity and cartilage degeneration in mouse destabilization of the medial meniscus (DMM) model of osteoarthritis. (a) N-104 was injected intra-articularly into DMM-operated joints twice weekly. Paw withdrawal threshold (PWT) was assessed weekly using the von Frey assay starting at week 2, and joints were collected for histology at week 8. (b) N-104 attenuated the DMM-induced decline in PWT of the ipsilateral joint. Data are presented as mean ± SD; n = 6 mice per group. *p < 0.05 by multiple t-tests at each time point; *n.s.*, not significant between sham and N-104 groups. **(c)** N-104 reduced cartilage degeneration, as shown by Alcian Blue/Picrosirius Red staining of the medial tibial plateau (MTP). Arrows indicate apoptotic or dead cells in the medial cartilage.