

## Effects of N5-1 Inhibition on IRF5 Signaling in Chondrocytes Versus Synovial Fluid Stem Cells

Daniella V. Davia; Haixiang Liang; Alex L. Chang; Pooja Swami; Henintsoa Fanjaniaina Andriamifidy; Betsy J. Barnes; Daniel Grande\*  
([DGrande@northwell.edu](mailto:DGrande@northwell.edu))

Orthopedics Research Laboratory, Feinstein Institutes for Medical Research, Northwell Health System, Manhasset NY 11030

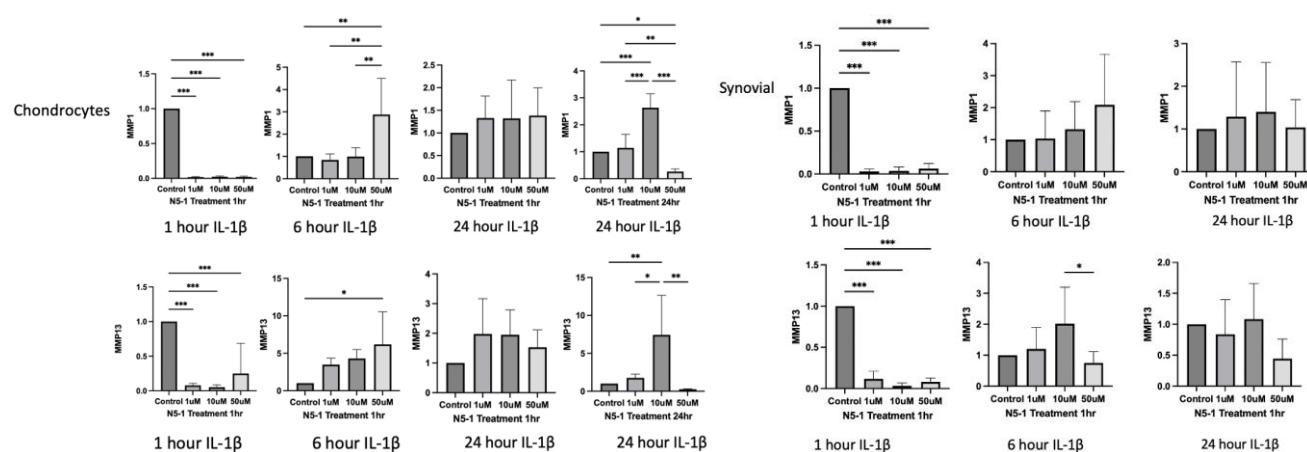
**Introduction:** Recent evidence suggests that the transcription factor interferon regulatory factor 5 (IRF5) may play a role in the pathogenesis of osteoarthritis (OA). IRF5 expression was previously shown to increase as the severity of OA increases [1]. The Barnes Lab developed the first inhibitor of IRF5, termed N5-1, that exhibited *in vivo* efficacy in three mouse models of lupus [2]. The purpose of this study was to determine if N5-1 could alter the biological function of IRF5 in an *ex vivo* inflammatory OA environment.

**Methods:** This experiment had two arms. The first arm consisted of the same treatment regimen for human chondrocytes and human synovial fluid stem cells (SFSC). Both cell types had a control group, three doses of N5-1 treatment (1μM, 10μM, and 50μM), and three different collection times of 1 hour, 6 hours, and 24 hours. All the groups excluding the control were first treated with N5-1 for one hour. After 1 hour, the N5-1 treatment medium was changed, and all groups were treated with 10ng/mL IL-1β until the respective collection time. The control group was treated with IL-1β for 24 hours. The second arm of this experiment consisted of human chondrocyte treatment with the different concentrations of N5-1 for 24 hours and then 10ng/mL IL-1β for 24 hours. After this treatment time, cells were harvested. Gene expression was measured by real-time PCR.

**Results:** Gene expression of IRF5, IL-6, IL-1β, MMP1, MMP13, ADAMTS4, IFNB1, MyD88, TLR3, TLR7, and TLR9 were compared among groups. For SFSCs and chondrocytes, MMP1 and MMP13 expression significantly decreased after 1 hour of IL-1β treatment, but then increased thereafter. In the 24-hour N5-1 treatment, chondrocyte MMP1 expression of the 50μM group remained significantly lower compared to the control group. However, at the same time MMP1 and MMP13 expression was significantly higher in the 10μM group. Most 6-hour timepoints showed significant increases of gene expression in the 50μM group of IL-6, IL-1β, IRF5, and MyD88 in SFSCs and chondrocytes. Chondrocytes also had increased ADAMTS4 expression at this point. In SFSCs, TLR3 expression was significantly higher in the 50μM group, while all other TLR genes showed non-significant changes. In chondrocytes, TLR7 expression significantly increased in the 10μM group at 6 hours, while all other TLR genes remained unchanged. In SFSCs, IRF5 expression was significantly higher in all N5-1 treated groups at 1 and 6 hours after the stimulation with IL-1β. In chondrocytes, this same trend was only shown in the 1μM group at 1 and 6 hours and the 50μM group at 6 hours post stimulation with IL-1β. Lastly, in the first hour, IFNB1 expression significantly increased in the SFSC 10μM group. This same trend was not shown in chondrocytes, but there was significantly higher expression of IFNB1 in the 50μM group with N5-1 treatment for 24 hours.

**Discussion:** The results of real-time PCR support the concept that the N5-1 peptide inhibits inflammation by post transcriptional regulation of IRF5. N5-1 works by binding to IRF5 protein and inhibiting its nuclear translocation. IRF5 nuclear translocation is a pre-requisite for it to bind to the promoters of target genes. Matrix metalloproteinases (MMPs) play important roles in the degradation of cartilage that causes progression of OA. Specifically, MMP1 and MMP13 are known to be expressed more in degraded cartilage [3]. After 1 hour of N5-1 treatment and 1 hour of IL-1β treatment, in both chondrocytes and synovial cells, the expression of MMP1 and MMP13 significantly decreased in the N5-1 treated groups compared to the control groups. However, after 6 hours the expression of these MMPs then increased. The increased expression of other genes, such as TLR3, suggest that other signaling pathways may be activated. This pilot study reveals that the N5-1 peptide can reduce inflammation in synovial cells. This trend was also seen in IL-6 expression within chondrocytes after 1 hour of IL-1β, but not in synovial cells.

**Significance/clinical relevance:** The results support that the N5-1 peptide is a potential short-term treatment to prevent cartilage degradation and that IRF5 is an upstream regulator of both MMP1 and MMP13 in SFSCs and chondrocytes. This is one of the first studies to link IRF5, OA, MMP1 and MMP13 together. In future studies, multiple treatments of N5-1 or a slow-release treatment should be tested to determine if N5-1 could have a longer lasting effect.



1. Ni, Z., et al., *The Role of Interferon Regulatory Factor 5 in Macrophage Inflammation During Osteoarthritis*. Inflammation, 2019. **42**(5): p. 1821-1829.
2. Song, S., et al., *Inhibition of IRF5 hyperactivation protects from lupus onset and severity*. J Clin Invest, 2020. **130**(12): p. 6700-6717.
3. Vincenti, M.P. and C.E. Brinckerhoff, *Transcriptional regulation of collagenase (MMP-1, MMP-13) genes in arthritis: integration of complex signaling pathways for the recruitment of gene-specific transcription factors*. Arthritis Research & Therapy, 2002. **4**(3): p. 157.