Senolytic Effect of Navitoclax on Chondrocytes and Synovial Fibroblasts in an Osteoarthritis Murine Model

Helen X.Tran[1], Brian O. Diekman[1,2]

[1]Joint Department of Biomedical Engineering, UNC and NCSU, [2]Thurston Arthritis Research Center, UNC at Chapel Hill,

bdiekman@email.unc.edu

INTRODUCTION

- Aging is the strongest risk factor in the development of osteoarthritis (OA).
- Cellular senescence has been identified as one of the main contributors to aging that occurs when cells enter a stable cell cycle arrest in response to external stress [1].
- This results in the accumulation of senescence-associated secretory phenotypes (SASPs), which are secreted from senescent cells and increase the severity of OA [2].
- Senolytic therapy has been used as a treatment for age-related diseases, that specifically induces apoptosis in senescent cells, thereby, reducing further production of SASPs.
- Prior studies have used navitoclax (nav) as a senolytic drug to selectively eliminate senescent chondrocytes, which has demonstrated high efficacy in OA-induced murine models *in vitro* [3].
- However, the senolytic effect of nav *in vivo* and on other OA-related cell types, such as synovial fibroblasts (SFs), still remain unknown.
- **Specific aims*:***
- (1) Evaluate senolytic effect of nav on synovial fibroblasts *in vitro*
- (2) evaluate senolytic effect of nav *in vivo* through intra-articular (IA) injection.

METHODS

1. Synovium profiling

- Murine synovium was isolated, digested, and analyzed using flow cytometry.
- Synovial fibroblasts (SFs) were stained with CD45, CD31, CD146, podoplanin (PDPN), and CD90.2 (Thy1).
- SFs were characterized as CD45-negative, CD31-negative, CD146-negative, and PDPN-positive cells.
- Within SFs, sublining was Thy1-positive, and lining was Thy1-negative.

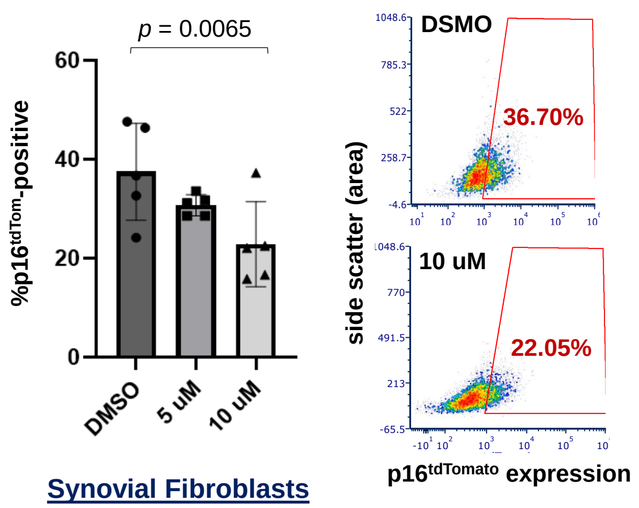
2. *In vitro* treatment of nav

- Cells from passage 2 of isolated SFs and passage 0 of isolated chondrocytes from knee cartilage.
- Control: DMSO 0.1%
- Treatment of nav for 48 hours
- Dosage: 5 uM and 10 uM

3. IA injection of nav

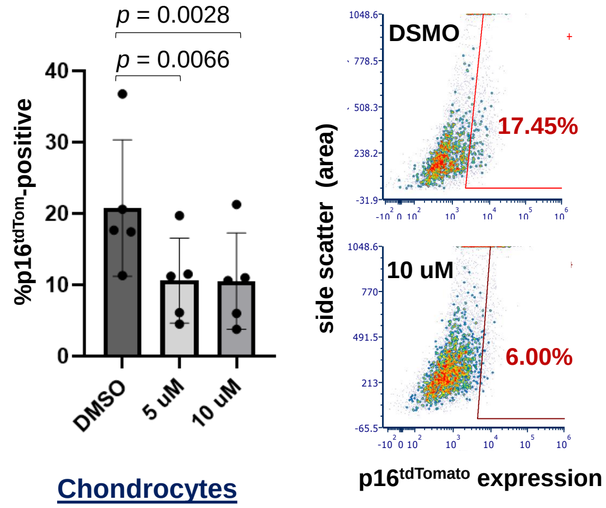
- The right hindlimb of each mouse was treated with nav.
- The left hindlimb of each mouse was injected with DMSO.
- A dose of 1 mg/ml nav was delivered to the joints through three injections.

IN VITRO SENESCENCE CLEARANCE



Synovial Fibroblasts

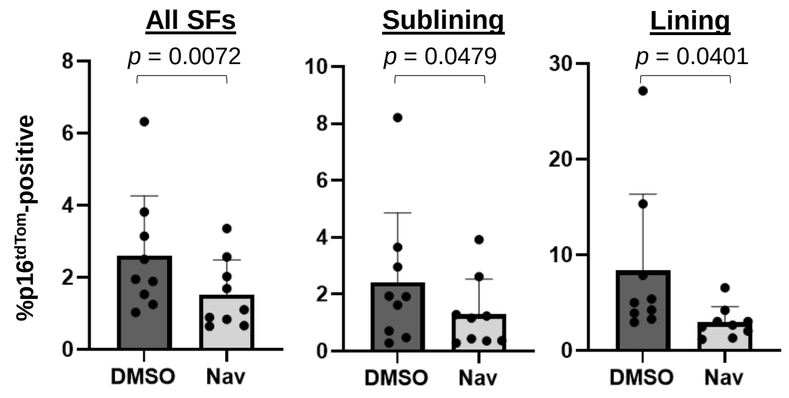
Reduction in percentage of senescent sublining fibroblasts, more significant in 10 uM of nav.



Chondrocytes

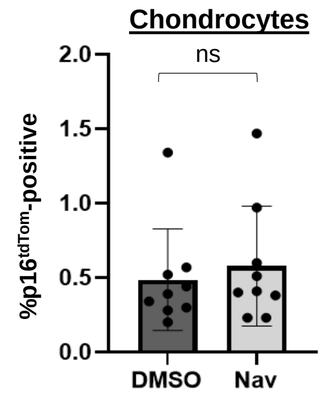
Reduction in percentage of senescent chondrocytes, no dosage effect.

IN VIVO SENESCENCE CLEARANCE



Percentage of p16-hi cells within the synovium

- Reduction of senescent cells were observed in all subpopulations within SFs.
- There was slightly more reduction in lining as compared to sublining, but lining also had higher $p16^{tdTomato}$ baseline.



Percentage of p16-hi cells within the articular cartilage

- No significant difference between DMSO control and nav treated groups.

DISCUSSION

Nav demonstrated senolytic effect by eliminating p16-positive cells in murine synovial fibroblasts both in monolayer and after intra-articular injection using p16tdTomato senescence reporter mice. However, nav only reduced senescence in murine chondrocytes *in vitro*, whereas, the *in vivo* clearance effect remains unclear. This could due to inefficient delivery of nav into the articular cartilage, and/or the lower baseline of senescent chondrocytes in physiological aging mouse model. Additional studies are required to assess the delivery of nav to articular cartilage using other methods besides IA injection, such as through microparticles.

REFERENCES

[1] C. Von Kobbe, "Targeting senescent cells: approaches, opportunities, challenges," *Aging*, vol. 11, no. 24, pp. 12844–12861, Nov. 2019, doi: 10.18632/aging.102557.

[2] O. H. Jeon, N. David, J. Campisi, and J. H. Elisseeff, "Senescent cells and osteoarthritis: a painful connection," *Journal of Clinical Investigation*, vol. 128, no. 4, pp. 1229–1237, Apr. 2018, doi: 10.1172/JCI95147.

[3] G. A. Sessions, M. E. Copp, J. Liu, M. A. Sinkler, S. D'Costa, and B. O. Diekman, "Controlled induction and targeted elimination of p16 ^{INK4a} -expressing chondrocytes in cartilage explant culture," *FASEB j.*, vol. 33, no. 11, pp. 12364–12373, Nov. 2019, doi: 10.1096/fj.201900815RR.